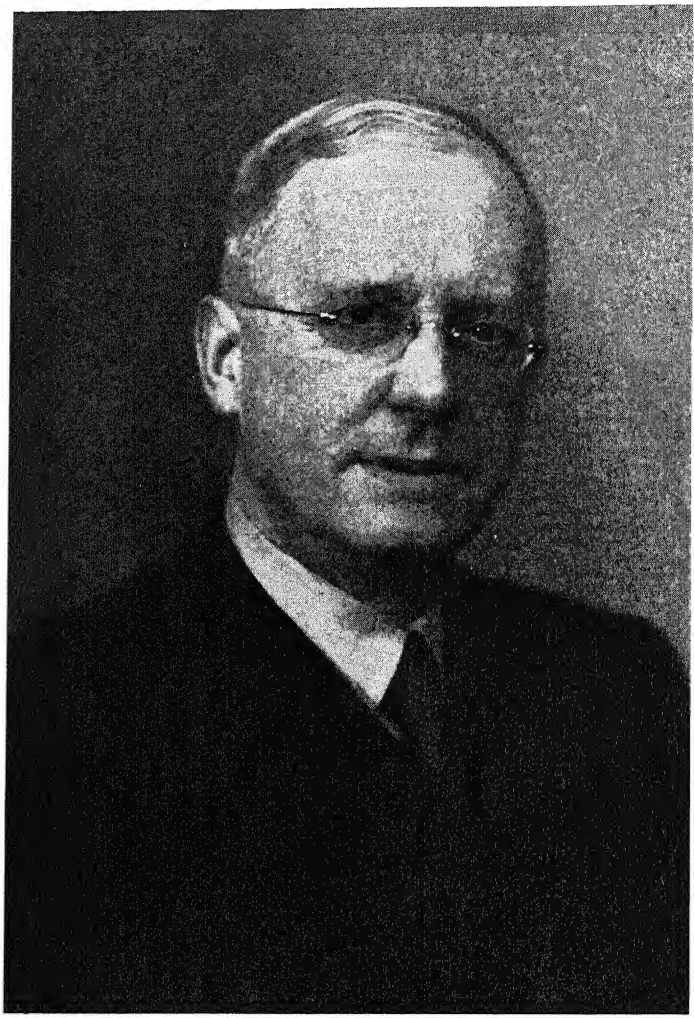


J. RAMSBOTTOM

THE BRITISH MYCOLOGICAL SOCIETY

By J. RAMSBOTTOM

The British Mycological Society was founded in 1896. After the Woolhope Club Forays had ceased in 1892, there was an attempt to make the Yorkshire Naturalists' Union an annual meeting-place for mycologists. As G. Massee and C. Crossland wrote in their account of the Yorkshire Foray of 1893: 'The justly celebrated Hereford Foray, which for many years monopolized the first week in October, and was the universally acknowledged meeting-place for exchange of opinion and courteous criticism between British and foreign mycologists, has unfortunately run its course, and it is the hope and ambition of the Yorkshire Union that the annual Yorkshire gathering may—by avoiding the weak points of its predecessor, which were mainly confined to an excess of hospitality—prove at least equally attractive and instructive to mycologists. In furtherance of this object no efforts are spared; a locality favourable for the object in view being selected, and an attractive, and it is to be hoped instructive, programme provided, embodying the leading current features of mycological interest, thus enabling everyone to become acquainted with the latest tendencies and discoveries in connexion with the subject.'

Though the Yorkshire Forays as such did not begin until 1891, many of the September Meetings of the Union, from 1881 to that date, were devoted to collecting fungi. A Mycological Committee was first formed in 1895 with the Rev. W. Fowler, a well-known naturalist and first President of the Union (1886), as Chairman.

M. C. Cooke and C. B. Plowright, both prominent Woolhopeans, attended several of the Yorkshire Meetings, as did also Carleton Rea, who had known the Hereford Meetings only when their palmy days were over. As the Yorkshire project was openly stated—it is mentioned by Massee in *Grevillea*, XXII, 47 (1893)—it had doubtless been freely discussed at the Yorkshire Meetings. There was some reorientation of ideas, however, for at Huddersfield in 1895, the formation of a National Mycological Union was formally discussed. A photograph taken at the Meeting is reproduced in the *Transactions*, V, pl. facing p. 182 (1915): it shows M. C. Cooke, C. Crossland, W. Fowler, G. Massee, J. Needham and C. Rea. James Needham was the old type of north-country working-man naturalist, an ironmoulder by trade, who thought nothing of a thirty-mile tramp over the moors in search of fungi and mosses. My friend, the late Prof. C. E. Moss, knew him well, and always spoke of him in the highest terms of praise and affection. The decision to form such a Union, with the title of the British Mycological Society, was taken at the Selby Meeting in the following year. George Massee was elected President; Charles Crossland, Treasurer; and Carleton Rea, Secretary.

Doubtless there were several factors which determined the decision.

Vol. XXXI, Parts III & IV: issued 29 July 1948.

Most field workers were domiciled in the Midlands and south of England; those north of the border had the Scottish Cryptogamic Society. It was also desirable that forays should be held in various parts of the country. Moreover, a publication was needed in place of *Grevillea*, which had stopped in 1894. Probably also there was no enthusiasm for the vaunted promise of austerity, or perhaps a lack of adjustment to the north-country temperament. Carleton Rea appears to have been the prime mover for a national society. He canvassed his mycological friends, and as he received considerable support pushed the idea to his utmost.

The Meeting at Selby was attended by M. C. Cooke, T. Hey, C. B. Plowright, C. Rea, and all the members of the Yorkshire Mycological Committee—U. Bairstow, T. Birks, W. N. Cheeseman, A. Clarke, C. Crossland, W. Fowler, G. Massee, J. Needham, J. W. Sutcliffe and H. T. Wager. Canon du Port was to have been there and to have read a paper, but was incapacitated by an accident. All present joined the Society and it began with 'about twenty'. A circular letter was afterwards sent to all those likely to be interested. The first Foray was held at Sherwood Forest in 1897. Massee was re-elected President, and Rea both Hon. Secretary and Treasurer, Crossland 'advocating the combination of these two offices in one as their division only entailed useless expenditure of the Club's funds'. The first number of the *Transactions* consisted of thirty pages and had a list of fifty members. Rea acted as Editor.

But a rift soon occurred. The 1898 Foray was held at Dublin, 'universal regret being expressed by all members that their President, Mr George Massee, F.L.S., was unable to attend the meeting'. The fact was that Massee and Rea had quarrelled. It is probable that Massee felt that as Secretary, Treasurer and Editor, Rea would have control of the Society and that his own influence would not be as great as he thought proper. Also there was a clashing of temperaments: as so often on such occasions, it was the points of disagreement that loomed large—Rea somewhat of a dandy, Massee a rough north countryman—both of rather violent temper, though their barks were usually more noticeable than their bites. Massee resigned from the Society and was accompanied in this self-denying ordinance by several of his Yorkshire cronies. In the circumstances it was the best thing that could have happened, though it might have wrecked the Society. C. B. Plowright was elected President, and Massee concentrated his attention on the Yorkshire Meetings. The next President was H. Marshall Ward. It was at the meeting at Rothiemurchus Forest Foray, over which he presided, that the 'Printing Fund' was begun. There had been a coloured plate of *Lactarius theiogalus*, drawn and hand-coloured by Mrs Rea, in the *Transactions* (vol. 1, pl. 5), and 'it was unanimously decided that an appeal should be made to the members to contribute voluntarily an annual sum in addition to their subscription, until the funds of the Society were sufficient to enable them to publish their *Transactions* with plates, which were necessarily costly, out of the annual income'. Marshall Ward was President for two years and Vice-President until his death in 1906. The success of the Society in its early years was due in large part to him, C. B. Plowright, W. L. W. Eyre and D. Paul.

So early as 1901 the scarcity of available copies of the *Transactions* began to be a problem, and it was decided that only the complete Volume 1, to be completed the following year, should be sold. In March 1902, there were sixty members.

J. W. H. Trail was President at the Hereford Meeting in 1902: 'at the last moment he was imperatively prevented from attending the meeting' but sent his address on 'Distributional Records', which dealt with detailed distribution mapping of flowering plants: it was not published.

At the Savernake Foray in 1903, presided over by W. L. W. Eyre, it was announced that the limit of 100 Foundation Members had been reached, of whom about seventy still remained on the active list. Worthington G. Smith, the President for 1904, was absent from the Whitby Foray, and provided no address. Haslemere first appeared as a Foray locality in 1905, with R. H. Biffen as President: since then it has been a favoured centre. Emile Boudier was elected the first Honorary Member.

An even closer approach to London was the Epping Foray in 1906, the President being Arthur Lister, who had revolutionized the study of the Mycetozoa. I regret never having met him, for he died two years later: his memory was held in high respect by all those who had known him, for his gracious charm and outstanding scientific ability and integrity.

Miss A. Lorrain Smith was President at Newcastle in 1907. She had begun that year the continuation of Crombie's 'British Lichens', but continued her interest in microfungi for many years.

The first of the informal Spring Forays was held at Shrewsbury in 1909. The Autumn Foray at Baslow in 1909 was one of the most outstanding of the series. It was presided over by M. C. Potter and was attended by the eminent French mycologists, René Maire, E. Peltereau, and E. Simon, known chiefly as an arachnologist. Over 530 species of fungi, ten new to Britain, and five new to science, and forty mycetozoa, were found. Rea described *Hygrophorus citrinus*, whereas Maire was responsible for *H. reai*, *Leptonia reaae*, after Mrs Rea, *Entoloma griseocyaneum* var. *roseum*, and *Omphalia alleni*, after W. B. Allen, a Shropshire naturalist, a great friend of Rea, an ardent collector of fungi and of mycetozoa: Rea often said that when he described a new species Allen always found it and made it really known: pressure of business prevented his attendance at many of the Forays in later years.

The following year, 1910, the Autumn Foray was at Wrexham with H. Wager as President. The list of fungi collected was small, but that of mycetozoa, forty-two species and two varieties, was the record for any Foray up to that date.

When I entered the service of the Trustees of the British Museum in 1910, Miss Lorrain Smith and Mr A. Gepp impressed on me the advisability of joining the Society, and my election dates from that year. I attended the Autumn Foray at Taunton in 1911 in company with A. A. Pearson, whom I had been coaching in botany and who had become interested in fungi. E. S. Salmon was President, and I remember sitting at the back of the room during his address, next to M. C. Potter, who was audibly in deep slumber. I knew nothing of the arrangements. The address

ended, the applause wakened Potter, who got on to his feet and proposed a vote of thanks with brilliance, though with criticisms that he would not have ventured upon if he had not been startled out of a sleep—though a sleep in which apparently he had heard more than I thought possible. E. W. Swanton gave a popular lecture, and I began a friendship with him and an interest in his work at Haslemere, which has lasted through the years. My friendship with Rea also started from those days, and at most of the Forays I was generally in his company in the field. He acted as referee and all brought their problems to him. He asked me to contribute something to the *Transactions*, and later sent the proofs of the next number for me to assist in correcting. The printing was done at Worcester and there were strange errors: one I remember was 'pins and needles' which I hesitated to alter to the more intelligible but prosaic 'pine needles'.

The Foray in 1912 was held at Aviemore, with Miss G. Lister as President. It lasted ten days and was the most successful Foray, from a mycological point of view, in my experience. The list of species of fungi was the largest ever obtained on a Foray, though it should be mentioned that the Foray lasted ten days and some members collected a few days before the Meeting. Even more remarkable was the list of Mycetozoa, which numbered eighty-two (including a new species): it was as if there was an assemblage to display their charms as a tribute to the knowledge and industry of our President. The Rev. D. Paul, eminent preacher and classical scholar, a mycologist of the old school, discussed fungi learnedly in the field, and old times over a stiff whisky indoors; W. D. Boyd, quiet and unassuming, read a somewhat dry paper on the fungus-flora of the Moray District, but was lively and instructive in his search, at one point stopping the brakes and bringing back *Sclerotium roseum*, which he had spotted from the appearance of the infected rushes, the origin of an annual demonstration at future Forays; Angus Grant, name, appearance and voice to match, came over the mountains to meet us with some magnificent specimens of *Boletus pinicola* and *B. sulphureus*—Rea used to say that Grant always found the finest specimens of the large rare Scottish fungi.

The Foray in 1913 was at Haslemere. It was there announced that Mrs Robinson had bequeathed £50 in memory of her late husband, John Branch Robinson, to be applied for the general purposes of the Society (subject to her mother's life interest). The Robinson Bequest Fund was instituted in 1914; it is always understood that it is to be used for providing coloured plates in the *Transactions*. A. D. Cotton presided.

By the time of the next Foray at Doncaster, war had begun, and for the first time one saw women knitting socks for soldiers during the meetings. A. H. R. Buller was President. Buller, like Marshall Ward, H. Wager and some other eminent mycologists, had no eye for species, though I doubt if he realized it. By temperament he was a born teacher, and immediately expounded whatever information he had acquired. He was at his best and happiest demonstrating some biological fact, as for instance at Doncaster, when he traced with abundant help, the mycelial strands of *Collybia platyphylla*, and at Keswick where he persuaded members to hold a séance in the dark to try to see luminosity evoked in leaves by fungal mycelium.

His patience on such occasions often tended to tire that of some members, but his boyish enthusiasm, his simple methods and his abundant results, delighted others. One could always draw him by suggesting that his explanations were teleological.

The Swansea Foray in 1915 was memorable for the excellent local arrangements made by H. R. Wakefield (Miss Wakefield's father); Mrs Rea presided.

E. W. Swanton was President at the New Forest Foray in 1916, but was unable to attend. C. H. Grinling, a non-practising clergyman and a great social worker at Woolwich, who in those days regularly came on Forays, was accompanied by F. Pethick Lawrence (now Lord): Somerville Hastings, also prominent later as a member of the Labour Government, attended, as he did whenever he could obtain respite from the duties imposed on him by his eminence as a surgeon. We were a small party. I remember how our nights were disturbed by the activities of a neighbouring bombing school, and how we enjoyed a dish of *Hydnum imbricatum*. Perhaps the Foray figures more prominently in my recollections because of its being the last before I went on active service.

Among the constant attenders at Forays about this period, was J. W. Ellis, who was keenly interested in microfungi, a medical man who died as a Lieut.-Colonel in the R.A.M.C. during the war while he was acting as the chairman of Medical Boards; Dr Bayliss Elliott, interested in Discomycetes, and her husband, W. T. Elliott, who specialized on mycetozoa, in which organisms Miss D. Cayley, Miss Hibbert-Ware, and later, Miss M. W. Rea, also collected assiduously, forming a slowly moving unit with Miss Lister as presiding genius; C. J. Sharpe, a retired tea-planter, who hunted for fungi as if they were game, and who always had a supply of pears for the ladies; H. H. Knight and R. Paulson, who quietly and assiduously collected lichens; N. Temperley, who always left his umbrella in the woods, and who was usually accompanied by A. Wallis, who specialized in photographing specimens, and W. N. Cheeseman, three greybeards fond of cider and a rest at some wayside tavern; Miss E. F. Noel, Miss C. A. Cooke and Miss K. E. Smith, who were all diligent collectors; N. G. Hadden, with a keen eye for rusts and mycetozoa; D. Mackenzie, a Scot, who was sufficiently interested in fungi and in the Society to make its meetings part of his annual holiday, which other members of the Scottish Cryptogamic Society, R. Barr, R. Johnstone, and Rupert Smith, though keener mycologists, could seldom manage; and J. Harvey Bloom, an old-fashioned type of country parson, a real John Bull in appearance, who had written on the flowers of Shakespeare, and who, after resigning his living, earned a livelihood by genealogical research. The average age of the members was considerably higher than now, for many had taken up fungi as a hobby in their retirement; consequently there was a continuous dropping out of those one looked forward to seeing. The programme was normally a gathering together of members on Monday and general conversation in the evening. There was a whole day's Foray on the Tuesday with the Annual Meeting in the evening. On the remaining days a late start was made to provide time for examining the finds, during which Mrs Rea was

always busy with her paintbrush: sometimes the pace was so forced that she was incited by her friends to rebel—but she was a willing 'slave', too keen on adding to her series of fungus paintings to regard 'Carl's' demands as unreasonable, and occasionally she would remain indoors to complete her task. The Presidential Address was delivered on Wednesday, most members dressing for dinner on that evening, if on no other. Usually Thursday evening was occupied by the reading of one or more papers, and on Friday it became the custom to have a commentary on the finds of the week, usually by Mr Rea. The long days in the fresh air tired most of the women, and they usually retired immediately after the meeting; many of the men, however, gathered together over drinks and smokes, some continuing to gossip and to reminisce until it was impossible to ignore the insistence of the hotel servant deputed to see to the extinguishing of the lights.

While abroad I was kept informed about the Society's affairs and gave a good deal of thought to its future. I was convinced that it could be developed into an organization worthy of our name and still retain the old club spirit, and wrote to that effect to several members.

Meanwhile, there was a move to broaden the basis on which the Society was run. As was to be expected, there had always been some criticism of Rea's supreme position, but though autocratic his was a most benevolent autocracy. Some members, grateful for the great services he had given to the Society, were anxious not to appear to be criticizing—others were somewhat scared of his possible reaction. The fact that he was busily engaged on a Tribunal of the Ministry of National Service provided an occasion for suggesting that he should be relieved of some of his burdens. At the meeting at Selby in 1918, three weeks before the end of the war, under the presidency of D. Paul, Rea was given the new office of General Secretary and elected an Honorary Member; Miss E. M. Wakefield that of Secretary, A. A. Pearson those of Treasurer and Foray Secretary, and Rea, Editor, with me as Assistant Editor—to be altered immediately to co-Editor. A council of the officers and four members was elected and commissioned to draw up a new set of rules. As all past Presidents were members of Council it seemed that we were a little megalocephalous, for our membership was only 156. Henceforth, until 1935, Miss Wakefield usually wrote the Foray reports. H. Wager was President for 1919, and thoroughly enjoyed the Council meetings where the revised Rules were drawn up. I had returned to England by the time the first meeting was held and having been out of the hurly-burly, perhaps saw more clearly what some of the pitfalls were.

At the meeting at Baslow later in the year, I had the feeling that many thought that words were enough—perhaps the reaction of still being out of things. A. D. Cotton, who had just paid a visit to the U.S.A., urged that the Society should take a more active part in the development of Plant Pathology in Great Britain, and suggested the formation of a special sub-committee. After a vigorous discussion in which one member suggested that the Society should be content with holding its Forays, the general opinion was that *all* branches of mycology were in its scope, as indeed was

stated in its Rules, and that this included pathogenic fungi. It was left to the Council to settle the details of any action. Having strong opinions about mycology, I expressed them and was at first much against the idea that we should adopt the suggestion then. However, some of us discussed the proposals, and agreed at a Council Meeting later in the year, that a sub-committee should be formed, comprising F. T. Brooks (Chairman), A. D. Cotton (Secretary), G. H. Pethybridge, J. Ramsbottom, the President and Secretary of the Society *ex officio*. The Baslow Meeting also decided to publish two numbers of the *Transactions* a year.

During the meeting a railway strike began. Under the threat several left, and when it eventuated many were stranded. [I had wangled 'compassionate leave', being still in the Army, and the getting back to duty added to the week's enjoyment.]

The Phytopathological Subcommittee (Committee 1930) served a very useful purpose as the Society increased in membership, for it helped to canalize the efforts and particular interests of many of the younger members of the Society. The chief point was that it gave an opportunity for plant pathologists to meet and discuss the various problems that faced them. Having been formed, what special tasks should it undertake? It was decided to prepare a 'List of common names of British plant diseases'. This was first published in 1929, a second edition in 1935, and a third as *List of Common British Plant Diseases* in 1945. The compilation was most carefully done, and many members and others gave every assistance.

A phytopathological excursion was arranged each year and from 1922 one of the London meetings was assigned to the Committee. The policy adopted from 1930 was to regard the Committee as a self-governing body: they elected their own Chairman and Secretary, and even their own members, the names submitted to the Annual Meeting being accepted without voting—a method rigidly adhered to until the Committee itself in 1943 suggested that its members should be formally elected.

Though the Committee has dealt mainly with subjects connected with the practice of plant pathology, often working in conjunction with the Association of Applied Biologists, it has also interested itself in aspects of mycology not strictly phytopathology, as it has in aspects of phytopathology outside the scope of mycology. This has been all to the good, for it has acted as a stimulus to those concerned with other branches of the study and has broadened the outlook of the practising plant pathologist.

It was out of a more far-reaching proposal of S. P. Wiltshire that compilation of the list of British species was undertaken; the several groups were undertaken by different members and publication of the remaining sections should not long be delayed now that circumstances permit of leisure for such work.

At the Minehead Foray in 1920, T. Petch was President. Rea, without a hint to anyone, proposed me as General Secretary. He told me afterwards that he had only held the post warm for me until I was back at the Museum.

At this meeting the subscription was raised from 10*s*. to £1. The two foreign honorary members, Boudier and Saccardo, had died since the last meeting, and N. Patouillard and R. Thaxter were elected. It was decided

to arrange a day's Foray for London students, to be preceded by a semi-popular lecture. The first student's Foray was held in October and the lecture was given by Somerville Hastings.

When driving back from Minehead with A. A. Pearson, he commented on the fact that I was beginning to have my hands full with extra-mural work, but that probably the post of General Secretary would not be much of a burden. He thought the Society was now well organized and that we had probably reached about the limit of our members. I demurred about the membership, and the years immediately following the first World War brought many new members. The list of members in September 1920 was 185; in August 1924, 344. There has been a gradual increase in membership since.

Miss Wakefield and I worked in close co-operation, she dealing with the more immediate internal affairs of the Society, while I concerned myself with more general matters. I felt that we needed more activity than was possible at the Spring and Autumn Forays, and the Annual Meeting at Keswick in 1922, with F. T. Brooks as President, agreed to the general outline of a programme much as we have it to-day: three meetings for the reading of papers, a phytopathological excursion and a day Foray for London students, one in conjunction with the Essex Field Club, and a third with the British Ecological Society; also that the *Transactions* should be published quarterly. The last remained an ideal rather than an accomplishment. In co-editing with Rea there was always a difference in policy. I assembled the *Transactions* and edited them. Rea held that papers should be published as accepted; any statement to which the editors disagreed could be commented on in a foot-note. My idea was to do all that was possible to make a paper presentable, and also to help the inexperienced in every way: sometimes this meant rewriting a paper. The policy succeeded except in attaining regularity, and occasionally there were murmurs from some who thought that this virtue was a cloak for every sin. Few I think realized the labour that such a procedure entailed. Usually Rea sent the manuscript to the printer within a day or two of receiving it—so we both carried on according to our lights.

I should like to acknowledge the great assistance I received from many members during the formative years. The help everyone gave and the spirit in which it was given are prominent in my happiest recollections. The names of those, other than officers, that stand out most clearly in retrospect regarding the Meetings and the recruiting of new members, are those of F. T. Brooks and W. Brown, but my former colleague, E. H. Ellis, was a very present help in other matters.

The programmes were always carried out, but the getting of contributions was often hectic to a degree: the notices not infrequently were completed at the printers! But the meetings were invariably a success—the most successful had not infrequently a doubtful origin. Investigators were encouraged to give preliminary accounts of their researches, and there was always full and helpful discussion, in that friendly, social atmosphere which has endeared the Society to many. We lived dangerously, we travelled hopefully. The meetings were held at University College, and we lunched

together in the Refectory, and often carried on with our discussions at tea.

The 1921 Meeting was at Worcester. It was our 25th Anniversary and, appropriately to time and place, our President was Carleton Rea. Always an excellent companion in the field, he delighted in displaying his old collecting grounds which he knew from practically every angle. It was agreed at the Meeting that we should purchase some reference books for use at the Forays, and the idea was mooted that we should form a library. This was eventually turned down after due consideration for, amongst other points, it would be necessary for the Society to have its own rooms with an increase in subscription.

Carleton Rea's *British Basidiomycetae* was published in 1922 'under the auspices of the British Mycological Society'. The arrangements for publication were undertaken by a committee consisting of A. D. Cotton, C. H. Grinling, A. A. Pearson and J. Ramsbottom. The period was one of difficulty, and the Cambridge University Press stipulated for a guarantee of £250. This sum was raised comparatively easily, but it was generally considered that the suggested price of two guineas was too high for students and so the main object of publication would not be achieved. The price was brought down to thirty shillings by all the guarantors agreeing to their guarantee being a donation.

Keswick, Windsor and Bettws-y-Coed stand out in memory as distinctly wet. The changing times may be judged from the fact that at Windsor I read a paper on 'Mushrooms and Toadstools' which I had broadcast from Savoy Hill a few days earlier.

1924 was the year of the Imperial Botanical Conference. A dinner was given to visiting mycologists in London during the Congress.

W. N. Cheeseman was President in 1925, but was unable to attend the Foray in Dublin, which, being the first visit of any scientific society 'after the trouble', was consequently the occasion of much social activity. In writing to me the President had sent a cheque for £100 'for the Printing Fund or any other purpose'. The Council decided to found a Cheeseman Fund for helping with the expenses of students otherwise unable to attend Forays—but before his approval could be obtained, Cheeseman died.

Hereford was visited in 1926. G. H. Pethybridge gave his address in Dr H. G. Bull's old house, standing between two rooms to do so. A dinner was given by the Woolhope Field Club in the dining-room at the Green Dragon, where the old Woolhopean dinners had been held, and the woodcut prepared for the 1877 dinner was used for the Menu Cards.* A. A. Pearson resigned from the Foray Secretaryship, and Miss Wakefield, who had drawn up the Foray Reports since 1918, undertook to make the arrangements for the meetings.

As more often than not our Forays are held in some district at the invitation of a natural history society, the reasons for our existence and for our visit, our proceedings and so on, are usually written up fully in the local press, but have received little mention in the London papers.

* This had also been used at the dinner given to overseas mycologists in 1924, and was again in service at the dinner which ended the Jubilee Meeting of the Society.

Hereford proved one of the few exceptions. I gave a public lecture there and having recently become possessed of a lantern slide of a photomicrograph showing the penetration of a *Gastrodia* tuber by the rhizomorph of *Armillaria*, showed it. There happened to be a travelling circus in the town that night, and, as was to be expected, some of our number preferred to form part of the audience there than at an elementary discourse; among them was F. T. Brooks—he was self-confessed. When commenting on the slide I said that it had been put in as I thought it would be of interest to some of our members, particularly to a certain learned member 'but—he has gone to the circus'. This aside got into the London press and so all over the country, with appropriate headlines such as 'Learned Professor prefers circus'!

In 1929 there was held the first Imperial Mycological Conference, and many of its delegates attended the Foray which that year was held at Bristol, with Miss Wakefield as a very proper President.

The Fifth International Congress was held in Cambridge in 1930. To mark the occasion Sir R. Biffen had been elected as our President. Several foreign guests attended the Whitby Meeting, and they were doubtless amused at a certain liveliness in our proceedings, which almost seemed a reaction to the sea air and to the restraint of the previous more august assembly. C. Rea retired from the Editorship, and B. Barnes and H. Wormald were elected co-editors with myself. It was at this Meeting that the Plant Pathology Subcommittee was reconstituted.

Of other Forays, that at Belfast in 1931, with A. A. Pearson as President, was notable for its social aspects. That at Haslemere in 1932, where Miss G. Lister presided, was one of the best attended. Mrs Rea's paintings made a good display in the new Educational Museum, and F. K. Sparrow was active amongst the aquatic Phycomycetes: I recollect instructing him thoroughly in a strongly recommended cold cure.

The much looked forward to Foray at Killarney in 1936, was most disappointing from a mycological standpoint, for many had hoped that intensive collecting would reveal the influence of the equable climate, a hope frustrated by the dry season. At the Meeting, presided over by F. G. Gould, Miss Wakefield announced her resignation from the Secretaryship. This was much regretted by all, for she had been so active in all our affairs since 1918, and was always helpful and friendly. We had worked together in the most harmonious way in building up the Society, which owed much of its smooth running to her influence. It was always easy for us to get into touch with one another, even out of official hours. C. G. C. Chesters was later appointed in her stead, and the fact that he was at Birmingham made some slight adjustments necessary. He expressed a wish to take over the programmes for the meetings, and this he did until 1939.

The Foray at Ludlow in 1937 provided its variation, for K. St G. Cartwright, the President, went down with influenza and though he could hardly be persuaded to do so, remained in bed while I read his Address.

At Aviemore, with Miss K. Sampson as President, we had a Foray which disappointed those who had recollections of abundant harvests in the neighbourhood, as well as those who had travelled wishfully so far north

for the first time. It had become obvious that the annual addition of the retiring President to the Council was making it too unwieldly in numbers, and that it was carrying a lot of dead wood: there was also a feeling that there might arise too great a tendency towards that state of mind attributed to the aged. K. St G. Cartwright had raised the question during his Presidency, and it was agreed to adopt his suggestion that the Council consist of the President and other officers, nine members (three to retire annually), and past Presidents previous to 1936. In future a retiring President became Vice-President for two years.

A meeting was arranged for Chipping Campden in 1939, but war broke out and the members of Council who were communicated with, agreed that neither the Foray nor the rest of the programme could be carried out. The Annual Meeting was held in December in the Linnean Society's Rooms (which never closed during the war) and E. W. Mason presided. The tea which followed was quite a festive occasion.

I had intended resigning from all offices in the Society, but as C. G. C. Chesters was tied to Birmingham with official and A.R.P. duties, I decided to carry on. We hoped to have some field meetings in 1940, but difficulties of travel, the occupation by troops of most suitable collecting grounds, and, later, the Blitz, made failure certain of any attempt to organize them: we were able however to hold two indoor meetings. The Annual Meeting was again held in December—as it has been ever since—but no tea was arranged for after H. Wormald's address. Rationing had not then become stringent, but it was thought that the probability of an air attack would make members anxious to get out of London: the opportunity to discuss matters and possibly, for some, the chance of seeing an air raid, made tea as crowded a function in a neighbouring café, as if it were an official affair.

In 1941 one of the indoor meetings was held in Cambridge—London suffered one of its worst air attacks during the previous night—and a token Foray was held at Epping Forest. W. C. Moore was President. Arising out of his Address, which reviewed the position of plant pathology in this country, several ideas which seemed appropriate to the changing times were discussed, some coming to fruition. C. G. C. Chesters resigned from the Secretaryship, not wishing to be a sleeping partner, and G. C. Ainsworth was elected in his place. T. Petch and E. M. Wakefield were elected Honorary Members. A two-day Foray was held in September 1942, with Royal Holloway College as Headquarters—half the College being occupied by the A.T.S.: Miss E. M. Blackwell was President. At the Annual Meeting it was decided to appoint a Foray Committee, a direct result of a recommendation made by a committee set up to study questions of systematic mycology. I resigned from the Editorship, for I was content that it was in good hands.

The Foray Committee arranged three day Forays in the neighbourhood of London in the following year, and got in touch with various local natural history societies. Mrs Mason acted as Secretary, and doubtless the Committee helped to stimulate many whose interest had been aroused by the prominence fungi had assumed during the war. An indoor meeting was held at Bristol, S. P. Wiltshire being President, in conjunction with the

Plant Pathology Field Day at Long Ashton. It now only remained for freedom of travel and freedom of movement to ensure that field work would proceed with renewed vigour. But this was not to come for another two years, and meanwhile the Annual Meeting continued to be held in London—in the Chelsea Polytechnic, 1944 and 1945, with R. W. Marsh and G. Smith as Presidents, because Dr Barnes kindly arranged for lunch in the refectory, a function much appreciated for several reasons, and because he permitted smoking in the Department.

In their Addresses during the war, the Presidents dealt to a greater or lesser extent with matters which appealed to them as needing consideration from their own particular standpoint. This acted as a stimulus, and the beneficial results of ensuing discussions cannot yet be calculated. Thus two reports were prepared, one on 'The need for encouraging the study of systematic mycology in England and Wales' (1944), the other on 'The teaching of mycology' (1946). These were circulated to all bodies likely to be interested. So far they have not been published, as it was thought better to get considered statements into the hands of competent authorities than possibly to antagonize them by distortions and exaggerations likely to result from a public 'campaign' at a time when every branch of science seemed likely to be advocated as a universal panacea for distempers in the body politic.

In 1945 I resigned the General Secretaryship as I had intended to do so soon as the war was over. I was elected Honorary Member, and, as a few minutes previously I had been elected President for the Jubilee Year, I felt that, even if I had outstayed my welcome, my parting had been graciously and magnanimously speeded.

In this mixture of history and reminiscence, I have tried to give a picture of the growth of the Society as I heard it from older members, and as I saw it, laying stress on the early development, less well known to many than recent happenings.

PAST-PRESIDENTS AND OTHER OFFICERS, MEMBERS OF THE COUNCIL AND HONORARY MEMBERS, TOGETHER WITH LISTS OF THE AUTUMN AND SPRING FORAYS 1896–1946

Presidents

G. Massee	Sept. 1896–8	O. V. Darbishire	1923
C. B. Plowright	1899	J. Ramsbottom	1924
H. Marshall Ward	1900–1	W. N. Cheeseman	1925
J. W. H. Trail	1902	G. H. Pethybridge	1926
Rev. W. L. W. Eyre	1903	(Sir) E. J. Butler	1927
Worthington G. Smith	1904	Dame Helen Gwynne-Vaughan	1928
(Sir) R. H. Biffen	1905	Miss Elsie M. Wakefield	1929
A. Lister	1906	Sir R. H. Biffen	1930
Miss Annie Lorrain Smith	1907	A. A. Pearson	1931
Carleton Rea	1908	Miss Gulielma Lister	1932
M. C. Potter	1909	W. Brown	1933
H. Wager	1910	B. Barnes	1934
E. S. Salmon	1911	M. Wilson	1935
Miss Gulielma Lister	1912	F. G. Gould	1936
A. D. Cotton	1913	K. St G. Cartwright	1937
A. H. R. Buller	1914	Miss Kathleen Sampson	1938
Mrs Emma A. Rea	1915	E. W. Mason	1939
E. W. Swanton	1916	H. Wormald	1940
Miss Annie Lorrain Smith	1917	W. C. Moore	1941
The Very Rev. David Paul	1918	Miss Elizabeth M. Blackwell	1942
H. Wager	1919	S. P. Wiltshire	1943
T. Petch	1920	R. W. Marsh	1944
Carleton Rea	1921	G. Smith	1945
F. T. Brooks	1922	J. Ramsbottom	1946

Vice-Presidents

Barnes, B. 1933, 1935
Biffen, Sir R. H., 1909–10, 1931
Blackwell, Miss E. M., 1943–4
Boyd, D. A., 1913
Brooks, F. T., 1923
Brown, W., 1934
Buddin, W., 1942
Butler, E. J., 1926, 1928
Cartwright, K. St G., 1936, 1938–9
Cayley, Miss D. M., 1939
Cheeseman, W. N., 1923
Chesters, C. G. C., 1943
Darbishire, O. V., 1924
Gould, F. G., 1935, 1937
Gwynne-Vaughan, Dame Helen, 1929
Ingold, C. T., 1946
Lister, A., 1907–8
Lister, Miss G., 1914–22, 1924–5, 1933
Marsh, R. W., 1945–6
Mason, E. W., 1940–1
Mason, Mrs U. C., 1945
Moore, W. C., 1940, 1942–3
Morgan, G., 1938
Paulson, R., 1928
Pearson, A. A., 1932
Petch, T., 1921, 1930–1, 1941
Pethybridge, G. H., 1927
Plowright, C. B., 1902
Potter, M. C., 1911–12, 1929
Ramsbottom, J., 1925

Vice-Presidents (continued)

REA, CARLETON, 1922, 1934, 1946
SAMPSON, Miss K., 1939–40
SMITH, Miss A. L., 1927, 1932
SMITH, G., 1944, 1946–7
WAGER, H., 1920
WAKEFIELD, Miss E. M., 1930, 1937
WARD, H. MARSHALL, 1903–6
WILSON, M., 1936
WILTSHIRE, S. P., 1944–5
WORMALD, H., 1941–2

General Secretaries

REA, CARLETON, 1919–20
RAMSBOTTOM, J., 1921–45

Secretaries

REA, CARLETON, 1896–1918
WAKEFIELD, Miss E. M., 1919–36
CHESTERS, C. G. C., 1937–41
AINSWORTH, G. C., 1942–

Foray Secretary

PEARSON, A. A., 1919–24

Treasurers

CROSSLAND, C., 1896–7
REA, CARLETON, 1898–1918
PEARSON, A. A., 1919–46

Editors

REA, CARLETON, 1896–1930
RAMSBOTTOM, J., 1919–42
BARNES, B., 1931–
WORMALD, H., 1931–45
MOORE, W. C., 1946–

Elected Members of the Council (1919–46)

AINSWORTH, G. C., 1937–9
ALLEN, W. B., 1920–1
BARNES, B., 1929–30
BARTLETT, A. W., 1934–5
BISBY, G. R., 1938–40
BLACKWELL, Miss E. M., 1940–1
BRETT, Miss M. A., 1939–41, 1944–6
BROOKS, F. T., 1921
BROWN, W., 1928–9
BUCKLEY, W. D., 1929–30
BUDDIN, W., 1926–7
BUNYARD, G. N., 1928–9, 1931
BUTLER, E. J., 1922–3
CAMPBELL, A. H., 1944–6
CARROTHERS, E. N., 1934–5
CARTWRIGHT, K. ST G., 1934–5
CAYLEY, Miss D. M., 1921, 1936–7
CHEESEMAN, W. N., 1919–20
CHESTERS, C. G. C., 1935–6
COOKE, G. J., 1937–9
COOPER, Miss C. A., 1932–3
CROXALL, H. E., 1946–
DARBISHIRE, O. V., 1922–3
DAY, E. M., 1930–1
DEACON, G. E., 1940–2
DENNIS, R. W. G., 1945–
DOBBS, C. G., 1945–
DOWSON, W. J., 1924–5
ELLIOTT, Mrs J. S. BAYLISS, 1919–20
ELLIS, E. A., 1943–5
FINDLAY, W. P. K., 1938–40
GARRETT, S. D., 1942–4
GOULD, F. G., 1926–7, 1932–3
GREEN, C. T., 1931–2
GREGORY, P. H., 1940–2
GRINLING, C. H., 1922–3
GWYNNE-VAUGHAN, Dame HELEN, 1923–4
HARRIS, R. V., 1941–3
HASTINGS, SOMERVILLE, 1923–4
HAWKER, Miss L. E., 1944–6
HOWARD, H. J., 1935–6
HUGHES, J. S., 1933–4, 1941–2
HUGHES, S. J., 1946–
INGOLD, C. T., 1943–5

Elected Members of the Council (1919–1946) (continued)

KNIGHT, H. H., 1927–8, 1935–6
MARSH, R. W., 1931–2
MASON, E. W., 1927–8
MASON, F. A., 1925–6
MASON, Mrs U. C., 1942–4
MOORE, W. C., 1936–7
MORGAN, G., 1936–7
MUSKETT, A. E., 1932–3
NOEL, Miss E. F., 1938–40
O'CONNOR, P., 1937–9
PAULSON, R., 1925–6
POTTER, M. C., 1919
PRESTON, N. C., 1941–3
REA, Miss M. W., 1939–41
SAMPSON, Miss K., 1930–1
SAMUEL, G., 1945–
SHARPE, C. J., 1924–5
SMITH, A., 1933–4, 1942–4
SMITH, Miss A. L., 1919
SMITH, G., 1942–3
SOWTER, F. A., 1946–
STEPHENS, Miss F. L., 1939–41
TOMKINS, R. G., 1931–2
WARE, Miss A. HIBBERT, 1920–1
WESTERN, J. H., 1943–5
WILKINS, W. H., 1933–4

Honorary Members

†BOUDIER, E., 1905–20
†BRESADOLA, G., 1921–9
DODGE, B. O., 1946–
FALCK, R., 1946–
GÄUMANN, E., 1946–
HEIM, R., 1946–
LISTER, Miss G., 1924–
MAIRE, R., 1939–
†PATOUILLARD, N. T., 1920–6
PEARSON, A. A., 1946–
PETCH, T., 1941–
RAMSBOTTOM, J., 1945–
†REA, CARLETON, 1918–46
†SACCARDO, P. A., 1916–20
†SMITH, Miss A. LORRAIN, 1924–37
†THAXTER, R., 1920–32
WAKEFIELD, Miss ELSIE M., 1941–

† Deceased

Forays

	AUTUMN FORAYS	SPRING FORAYS
1897	Worksop, Notts	—
1898	Dublin, Eire	—
1899	Lyndhurst, Hants	—
1900	Boat of Garten, Scotland	—
1901	Exeter, Devon	—
1902	Hereford	—
1903	Marlborough, Wilts	—
1904	Whitby, Yorks	—
1905	Haslemere, Surrey	—
1906	Chingford, Essex	—
1907	Newcastle-on-Tyne, Northumberland	—
1908	Drumnadrochit, Scotland	—
1909	Baslow, Derby	Shrewsbury, Salop
1910	Wrexham, Denbigh	Chester, Ches
1911	Taunton, Somerset	Barnard Castle, Co. Durham
1912	Forres, Scotland	Worcester
1913	Haslemere, Surrey	Dolgelly, Wales
1914	Doncaster, Yorks	Symonds Yat, Heref.
1915	Swansea, Wales	Baslow, Derby
1916	Lyndhurst, Hants	—
1917	Shrewsbury, Salop	—

Forays (continued)

	Autumn Forays	Spring Forays
1918	Selby, Yorks	—
1919	Baslow, Derby	—
1920	Minehead, Somerset	Painswick, Glos
1921	Worcester	Haslemere, Surrey
1922	Keswick, Cumberland	Norwich, Norfolk
1923	Windsor, Berks	Bristol, Glos
1924	Bettws-y-Coed, Wales	Matlock, Derby
1925	Dublin, Eire	Tintern, Mon
1926	Hereford	Arundel, Sussex
1927	Aviemore, Scotland	Marlborough, Wilts
1928	Littlehampton, Sussex	Oxford
1929	Bristol, Glos	Petersfield, Sussex
1930	Whitby, Yorks	King's Lynn, Norfolk
1931	Belfast, Northern Ireland	Horsham, Sussex
1932	Haslemere, Surrey	Ludlow, Salop
1933	Newcastle-on-Tyne, Northumberland	Lyndhurst, Hants
1934	Norwich, Norfolk	Stroud, Glos
1935	Totnes, Devon	Matlock, Derby
1936	Killarney, Eire	Tunbridge Wells, Kent
1937	Ludlow, Salop	Beccles, Suffolk
1938	Aviemore, Scotland	Chipping Campden, Glos
1939	—	Arundel, Sussex
1940–5	Except for a two-day Foray in the Englefield Green (Surrey) area in September 1942 and three successive day Forays at Haslemere in September 1945, only day Forays were held during these years.	
1946	Whitby, Yorks	Wheatfen Broad, Norfolk

THE JUBILEE MEETING, LONDON, 20–25 OCTOBER 1946

The Fiftieth Anniversary of the founding of the Society was celebrated during 20–25 October 1946 by a series of meetings culminating in the Fiftieth Annual General Meeting, the Presidential Address, and five paper-reading sessions in the rooms of the Royal Institution, Albemarle Street, London on Wednesday, Thursday, and Friday, 23–25 October.

By the generous help of the British Council these celebrations were attended by the following mycologists from abroad: Prof. N. F. Buchwald (Denmark), Dr L. Doyer (Holland), Dr C. W. Emmons (U.S.A.), Prof. R. Falk (Palestine), Dr Nils Fries (Sweden), Prof. E. Gäumann (Switzerland), Prof. R. Heim (France), Prof. J. Kochman (Poland), Prof. P. Martens (Belgium), Prof. J. B. E. Melin (Sweden), Prof. J. A. Nannfeldt (Sweden), Dr A. J. P. Oort (Holland), Dr F. Petrak (Austria), Dr A. Pilat (Czechoslovakia), and Prof. A. E. Traaen (Norway).

On Sunday, 20 October, there was an excursion for the foreign guests and members to Kew Gardens and Hampton Court. The party assembled at noon on Kew Green where they were welcomed by Dr W. B. Turrill, Keeper of the Herbarium, who led a tour through the gardens. After a brief visit to the Herbarium, the party proceeded by motor coach to Richmond for lunch, and then drove on to Hampton Court, where the gardens and the royal apartments were visited.

Next day the visitors availed themselves of arrangements that had been made for visiting the Herbarium of the Royal Botanic Gardens and the Imperial Mycological Institute at Kew, the Department of Botany of the British Museum (Natural History), the Linnean Society of London, the London School of Hygiene and Tropical Medicine, Rothamsted Experimental Station and the Ministry of Agriculture's Plant Pathology Laboratory at Harpenden, the John Innes Horticultural Institution, or the East Malling Research Station.

At 5.30 p.m. on the same day the British Council held a reception at its Visitors' Department in Brook Street, where the guests were received by Prof. Scott Watson and the President of the Society.

Later in the week visits were also made to an exhibit of mycorrhiza arranged by Dr M. C. Rayner at Bedford College, and interesting features of the Linnean collections were demonstrated to a small party by Mr S. Savage, Assistant Secretary of the Linnean Society. On Thursday the foreign visitors were guests of the Linnean Society at one of its ordinary meetings and on Friday they, and the Organizing Committee, were entertained to tea by Dr and Mrs Ramsbottom at the Visiting Scientists' Club in Old Burlington Street.

Tuesday, 22 October, was devoted to a Foray in Windsor Forest and a large party assembled for the purpose at noon at the Royal Holloway College, Englefield Green. Heavy rain prevented a start until after lunch,

but a number of interesting finds were made in the Clockcase Plantation and adjacent parts of the forest and the collections were displayed in the Botanical Laboratories of the College, kindly placed at the Society's disposal by Miss E. M. Blackwell.

Proceedings of the Annual General Meeting

The Fiftieth Annual General Meeting was held in the rooms of the Royal Institution on Wednesday, 23 October 1946 at 10 a.m., with the President, Dr John Ramsbottom, O.B.E., M.A., in the chair.

The meeting was opened by the transmission of the following telegram to The King:

> 'The President, Council, and Members of the British Mycological Society assembled in general meeting on the occasion of the Fiftieth Anniversary of the foundation of the Society tender to His Majesty the King an expression of their loyal greetings and humble duty.'

His Majesty was pleased to reply:

> 'The President, the British Mycological Society.
>
> The King would be grateful if you would express to the Council and members of the British Mycological Society His Majesty's sincere appreciation of the loyal message which was addressed to him on the occasion of the 50th anniversary of the Society's foundation.'

After the Minutes of the previous Annual General Meeting had been read and signed the Secretary read messages of greetings which had been received from the Woolhope Naturalists' Field Club, the Mycological Committee of the Yorkshire Naturalists' Union, the Botanical Section of the Leicester Literary and Philosophical Society, and the Mycological Society of America. Subsequently, congratulations were received from the Society for Chemical Industry. The delegates from Holland brought a message of goodwill from the Nederlandsche Mycologische Vereeniging. Prof. N. F. Buchwald presented the Society with an illuminated address from the Society for the Advancement of Mycology in Denmark, and Dr A. Pilat presented a copy of his two-volume monograph on the Polyporaceae to the Society's library. Mr Herbert Morrison, Lord President of the Council, sent a letter expressing his interest in the activities of the Society. Apologies for absence and greetings from several past presidents and from a number of members in this country and overseas were also noted.

In welcoming the guests the President said that in view of the fact that our Society had attained its Jubilee, the Council, with the unanimous approval of members, had decided that the Anniversary Meeting should be made the occasion for stressing the importance of our studies. An organizing Committee was formed consisting of the Secretary, Prof. C. T. Ingold, Messrs W. C. Moore, G. Smith and himself. The Committee was

convinced that it would be beneficial to the study of mycology if the enveloping fog resulting from the war could be penetrated and to some extent dispersed. The British Council was approached and enthusiastically supported the scheme put forward. Meanwhile, the programme for a three-day celebration was drawn up. The British Council funds would allow of only one representative from each country except France, and could not cover hospitality for Dominion or Colonial regions, or for the U.S.A. However, the British Council agreed to make all arrangements for the Society's guests, and to regard them in every way as official guests. As the invitations were for a week a programme was arranged for the guests which it was hoped would interest them.

The special evening meetings were solely for the purpose of allowing for the free intermingling of guests and members, impossible at the daily sessions. The Council held strongly the view that no formalities were necessary. Our guests were our guests. We were happy to welcome them. We should gain much from their presence and we hoped that the benefit would be mutual. He trusted that it would become our custom to give special invitations to one or more foreign mycologists to attend our autumn Forays. An exchange of ideas on the constituents of our fungus flora would be of inestimable benefit.

In this our Jubilee year we had to mourn the loss of Mr Carleton Rea, who was mainly responsible for the founding of the Society and who ran it practically single-handed for twenty years; he was proud of its achievements, and he had looked forward to being here. To the older members he was a friend—to some of them a close friend—to new members a tradition. But he was ever ready to give of his unrivalled knowledge of our fungus flora both to the expert and to the beginner.

Other deaths we had to record were those of Capt. F. Gardner, which occurred in the Channel Isles in 1941 (he joined the Society in 1898), Prof. H. Chaudhuri, Dr M. Nierenstein, Miss M. E. Notley, and Mrs S. Pershouse. Reviewing the past year, as far as it was possible to do so, the President drew attention among other things, to the circulation in June, to Universities and other interested bodies, of a *Report on the Teaching of Mycology* which had been prepared at the direction of the Council by a Subcommittee consisting of Miss E. M. Blackwell, Prof. C. G. C. Chesters, Dr Lilian Hawker, Prof. C. T. Ingold (Chairman), Dr J. Ramsbottom, and the Secretary.

He knew that when the Society attained its centenary his successor would view the activities of the next fifty years with as much satisfaction as he did those of the last fifty, but he doubted whether that successor would be more proud than he was of having been chosen President on such an auspicious occasion.

The Treasurer, Mr A. A. Pearson, then submitted his Annual Statement and the accounts were adopted.

The following Officers and Members of the Council for 1947 were elected: *President*, C. G. C. Chesters; *Vice-Presidents*, J. T. Duncan, in addition to the two past presidents, J. Ramsbottom and G. Smith; *Secretary*, G. C. Ainsworth; *Foray Secretary*, G. Smith; *Treasurer*, W. Buddin;

Editors, B. Barnes and W. C. Moore; *New Members of Council*, C. E. Foister, W. D. Graddon and C. J. Hickman (to replace Miss M. A. Brett, A. H. Campbell and Miss L. E. Hawker). Dr Mary Glynne, R. Hull and I. F. Storey were elected as new members of the Plant Pathology Committee.

The President moved from the Chair that Dr B. O. Dodge (New York), Prof. Richard Falk (Palestine), Prof. Ernst Gäumann (Zurich), Prof. Roger Heim (Paris), and Mr A. A. Pearson (who had been Treasurer of the Society for twenty-eight years) should be elected as Honorary Members. This was agreed to with enthusiasm.

Thirty-seven new Members and five new Associates were elected making a total of sixty-two new Members and fifteen new Associates for the year.

After the programme for 1947 had been briefly discussed the meeting adjourned until 11 o'clock when the President delivered his address entitled 'Mycology then and now'. A vote of thanks to the President for his address, proposed by Dr Nils Fries and seconded by Dr R. V. Harris, concluded the meeting.

Paper-Reading Sessions

The papers read at the five sessions and printed in full in this volume were designed to show the diverse relationships of mycology. They were grouped as follows:

Medical mycology; Mould products

Chairman: Sir Alexander Fleming, F.R.S.

C. W. Emmons	Medical mycology (p. 40)
J. H. Birkinshaw	Some aspects of fungal metabolism; with particular reference to the production of antibiotics (p. 50)

Seed-borne fungi

Chairman: W. C. Moore, M.A.

Miss L. Doyer*	Several seed-borne fungus diseases and methods for identifying them in seed testing (p. 67)
A. E. Muskett	Technique for the examination of seeds for the presence of seed-borne fungi (p. 74)
Miss Mary Noble	Seed-borne diseases of clover (p. 84)

Mycorrhiza; Soil fungi

Chairman: The President

J. E. B. Melin	Recent advances in the study of tree mycorrhiza (p. 92)
C. G. C. Chesters	A contribution to the study of fungi in the soil (p. 100)

To conclude this session the film *Nématodes prédateurs*, made by J. Comandon and P. De Fonbrune of the Pasteur Institute, was shown.

* After reading her paper Dr Doyer presented the Society with an inscribed copy of her *Manual for the Determination of Seed-borne Diseases*, 1938.

Growth factor requirements of fungi

Chairman: Prof. W. Brown, F.R.S.

Nils Fries	The nutrition of fungi from the aspect of growth factor requirements (p. 118)
Miss L. E. Hawker	The effect of certain growth substances on mycelial growth and fruiting of *Melanospora destruens* Shear (p. 135)

A discussion followed in which Dr B. C. J. G. Knight, Miss M. A. Keay, Mr G. Samuel, Dr W. G. Keyworth, Dr A. J. P. Oort, Prof. E. Gäumann, Dr J. Ramsbottom, and Dr C. W. Emmons took part.

Taxonomy

Chairman: The President

Miss M. A. Brett	The problem of *Cladosporium herbarum* (p. 141)
Miss E. M. Wakefield	Taxonomic problems in Hymenomycetes (p. 152)
Roger Heim	Phylogeny and natural classification of macrofungi (p. 161)

Informal Evening Meetings

On Wednesday and Thursday evenings exhibits were displayed in the staff common-room of the London School of Hygiene and Tropical Medicine, and a short informal meeting was held on each evening.

On the first occasion Dr W. A. R. Dillon Weston exhibited specimens of his skilfully made glass models of macro- and micro-fungi and Dr E. J. H. Corner showed a selection of drawings for a monograph on *Clavaria* which he had executed while held prisoner by the Japanese at Singapore. The meeting took the form of a short film show at which Dr P. H. Gregory introduced slow-motion films, made by Mr E. D. Eyles, of a spore-dispersal mechanism of *Lycoperdon perlatum* and the effect of drops of water falling on a pustule of *Nectria cinnabarina*.

The second evening was devoted to an exhibit of photographs of the past presidents, and other items illustrating the Society's history, while the President commented on a series of lantern slides of cartoons by Worthington G. Smith of nineteenth-century fungus Forays and of photographs of the Society's Forays. Among the exhibits the holograph manuscript of Carleton Rea's *British Basidiomycetes*, now deposited in the British Museum, attracted attention.

On the last evening a dinner was held in the Refectory of the School, when eighty members and friends were present. The menu cards had been printed from the original wood-block engraved by Worthington G. Smith, with characteristic mycological humour, for the 1877 Foray dinner of the Woolhope Club. In conclusion there were speeches by the President, Dr C. W. Emmons, and Dr Nils Fries.

PRESIDENTIAL ADDRESS

By J. RAMSBOTTOM

It devolves upon me now to deliver an address as President. It is the British custom that the President of a Society, at some time during the period of his presidency, shall deliver an address. I understand that it is not customary in most other countries, but I have heard that, like many of our insular peculiarities, it is not without its foreign admirers. In our own Society it serves a very useful purpose both for the body politic and for the individual. It is our practice, so far as is expedient, to choose as our Presidents, members who represent different aspects of mycology. A President has free and unrestricted choice of subject for his address. He may elect to give a reasoned statement of his particular interests, or he may put forth ideas and suggestions that he would have little other opportunity of publishing. In our valuable series of Presidential Addresses we have had many useful summaries, and frequently matters have been aired which have had a beneficial effect on the management of our Society and even on mycology in general.

A Presidential Address is not open to discussion unless there are special circumstances. This will explain the absence of any open expression of disagreement or criticism of any remarks I may happen to make. This may deprive our immediate proceedings of a certain liveliness, but, in spite of this, I shall endeavour not to be unduly provocative.

The occasion of the Fiftieth Anniversary Meeting of the British Mycological Society seemed to its members sufficiently noteworthy to warrant some special activities. It was considered that our celebrations could best take the form outlined in the programme, with two main objects in view, the one an endeavour to indicate the prominent part fungi play in so many important matters, the other an attempt to take up again the broken threads of friendly international relations which require full play for our science no less than for the general good.

The British Mycological Society has done me great honour by electing me President for its Jubilee year. I wish to express my gratitude and appreciation to the members of the Society, a Society for which I have long had a feeling which is probably best expressed as affection—for it has unusual characteristics. On behalf of the members it is my great privilege to offer a warm welcome to our visitors, and more particularly to our many foreign guests. We trust that they will enjoy to the full their stay in this country, and that they will carry away happy memories, both mental mycologically and spiritual mycologistically. Unfortunately it is not in our power to offer them the solid and liquid bodily comfort which would have fallen to their lot in more spacious times, nor could we avoid the heteroecism* to which they have been subjected. Many here to-day, have walked

* There was, of necessity, a change in their hotel.

through the valley of the shadow of death during the past few years, and for some of our visitors the way has indeed been long and dark. The fact that so many have been able to accept the invitation to join us in our celebrations may surely be taken as a sign that we are emerging into the green pastures of civilization.

It is owing to the good offices of the British Council that we were able to extend so many personal invitations and we wish to thank those of the Council who have given so much of their time and experience to helping us.

Perhaps one further remark is necessary. The word 'British' may seem a little prominent in these preliminary remarks. May I say that though we look forward to benefiting mycology in this country through the inspiration of such an invasion by a type of foreigner we shall always welcome with a special kind of warmth, we should count our intentions incompletely fulfilled if our guests did not benefit to the full, not only from contacts with us, but amongst themselves. The friendly discussions during the jaunts preliminary to our more serious business, should bear fruit not only during the present meeting, but through the years of steadfast endeavour needed to repair the ravages of recent calamities.

It will doubtless have been noticed that pure taxonomy does not figure unduly largely in the sessions that are to follow. Indeed it is purely by chance that one session is devoted to it, for as originally planned, this was intended to be for general papers; the papers available covered a wide range—those eventually chosen grouped themselves naturally. What might have been regarded by some as a serious omission thus remedied itself, but the programme as a whole must not be judged as showing the relative values placed by the organizing committee on the different branches of mycology; the programme was planned with a view to indicating some of the ramifications of the study rather than its content. All these matters, however apparently remote, play a part in the efforts to secure a firm foundation for taxonomy and a reasonable superstructure. This, which implies that we should endeavour to learn all we can of the *nature* of the organisms we wish to classify, is a view which is not quite so unpopular as it was fifty years ago. For myself it has strengthened through the years until I regard it as a truism. It is best stated in the words of Asa Gray, which startled me when I read them as an undergraduate, and which I quoted the last time I occupied this chair, twenty-two years ago. 'Botanical classification, when complete and correct, will be an epitome of our knowledge of plants.' It would be well to display this as a text not only in every herbarium, but in every University Botanical Department. It is a dignified statement. The other aspect of systematic mycology, that of identification, does not lend itself to so sonorous a sentence. One would have thought that the correct identification of a fungus, or any other organism which was the subject of profound research, would be considered as important as any part of the investigation. In spite of confusions and controversies which have constantly and needlessly taken place because of failure to heed it, there is still a tendency towards casual identification. It seems so obvious that one would say it was only common sense—though this has been stated to be the rarest of all the senses. Perhaps the best form

here would be a slogan such as 'Make sure what you are talking about'.

Our foreign guests must bear with me if I lay stress on the condition of affairs in this country. The choice of a subject for my remarks presented many difficulties. The edifice is spacious, the occasion is festive, and there is a loaded table of meats in front of us, so it has seemed best to leave these matters for later Presidents; we are indeed fortunate in the number of members well worthy of filling the position.

The title 'Mycology then and now', is sufficiently wide and indefinite to enable me to play the part of an elder statesman, which seems to have been thrust upon me through the lapse of years, and by my fellow members who have pointed out that I have been closely connected with mycology for more years than most, more generally than most and more particularly with the Society than most. This being so, I feel free to express certain opinions regarding mycology in this country, which youth, shackled by expediency, might—possibly—have considered impolitic on an occasion such as this.

The British Mycological Society began as a club with the main object of holding Forays in different parts of the country. I shall have occasion to refer to this in more detail on Thursday evening, but it would be inappropriate not to mention that the chief mover in its formation was Carleton Rea, one to whom the Society generally, many of us particularly, owe much: it is a great sorrow to us that he died earlier in the year, for we had looked forward to his taking an active and characteristic part in the present celebrations.

To understand the position of mycology in 1896, it is necessary to go back a few years. As in most countries, our early floras treated fungi as an appendage, if at all. With few exceptions there was no real interest taken in them. James Bolton published *An History of Fungusses growing about Halifax* in three volumes, in 1788, with two supplements in 1791. It was the first British work entirely devoted to fungi, but neither the descriptions nor the plates are of outstanding merit. James Sowerby's *Coloured figures of English Fungi*, was the next wholly mycological work. The plates are excellent, for Sowerby was an outstanding portrayer of natural history subjects. The work was essentially produced as a means of selling plates, and was one of a series which included *British Mineralogy*, *Mineral Conchology of Great Britain*, and *English Botany*. The descriptions are for the most part very poor and contrast sadly with those of *English Botany*, which were written by Sir James E. Smith. Sowerby was really interested in fungi, and made a series of painted pipe clay models which he exhibited at his Museum at Lambeth in an attempt to educate the public to a knowledge of edible and poisonous species. Moreover, he wrote a very sensible report on the notorious ship of H.M. Navy, the *Queen Charlotte*, which by unseaworthiness through dry rot, had brought this perennial scourge of our fighting ships as prominently before the public as it was in the days of Samuel Pepys.

Thomas Purton in his *Midland Flora*, 1817–21, gave good descriptions and drawings of many species, and Robert Kaye Greville in his better known *Scottish Cryptogamic Flora*, 1823–8, proved himself a mycologist of the

first rank, but later spent all the time he did not devote to the study of diatoms, in trying to alleviate social evils. He was the mycological mentor of M. J. Berkeley, who for over half a century, from 1836 till 1889, was the recognized leader in British mycology. Berkeley's rise to fame was meteoric. He swung over from mollusca, microscopic algae and mosses, chiefly because W. J. Hooker had asked his help in preparing the fungus section of the continuation of Smith's *English Flora*; his assistance was so avid that he was asked in 1832 to undertake the treatment of the agarics—then, in 1834, of the whole of the fungi. The manuscript was delivered the following year and published in 1836. Immediately, Berkeley became the recognized British authority. His work was based on Fries's *Systema Mycologicum*, but it is full of original observations. Unlike Fries, Berkeley paid great attention to microscopic detail, though he first used a simple microscope, then doublets, and did not possess a compound microscope until 1868. It will be remembered that he was one of those who made out that the quaternary arrangement of spores was characteristic of the Hymenomycetes as a whole, and not restricted to a few species, and by describing the basidium in its several forms, provided the criteria for the main divisions. He extended the observation to the Gasteromycetes. Fungi in all their manners and mannerisms became Berkeley's preserve. A century ago when the Potato Murrain caused the Irish famine—and incidentally the fall of Sir Robert Peel—Berkeley was called in by the Commission appointed by the Government, and it is from his statement that a fungus was the cause of the disease and did not result from it, that the study of plant pathology may be said to have begun in this country.

Throughout an active mycological career, in time mostly occupied in responsible ministerial duties or in the effort to add to the stipend he found inadequate for maintaining his large family, Berkeley, in addition to describing many collections from abroad as well as constantly working at British fungi, did more than anyone of his period to show the importance of fungi as organisms. He played an important part in the studies on cholera, which many supposed was a fungal disease; he was frequently consulted about tropical diseases, and succeeded in convincing medical opinion in this country that the strongly supported and widely advertised claims of Hallier on the pleomorphism of fungal disease organisms were sheer drivel. If Berkeley's sane views about medical fungi had been taken as a model by later writers in this country, the subject would not now be in its present backward state, one might even say disrepute.

Berkeley, as a country clergyman, was without any real influence on the study of mycology in the sense that he could impose his opinions on those who were to receive or those who bestowed posts. He was a friend of many influential botanists, but the field was left to him.

During the last 100 years in this country, Botany, writ large, has been a matter of fashion and cliques. When Berkeley was in his prime the old style all-round botany as epitomized by Robert Brown, had given place to purely descriptive systematics. However, in the early eighties it became the fashion to study in Germany, and what was called the 'new botany', anatomy and physiology, gradually came to the fore and systematic botany was

eventually forced out, and now it receives scant academic recognition. Palaeobotany, fern anatomy and physiology ruled for a time; then cytology, ecology and genetics. As this is an age of specialization, the study as a whole tends to suffer from the myopic view of the coterie which happens to be in the saddle, for dog doesn't eat dog and many of the prizes are the rewards for adhesion to the sects.

Mycology has never been on the map, though the period of its sojourn in the wilderness is rapidly approaching its end. It is difficult to account for the attitude shown by many towards the proper teaching of the subject. Prominence is given to the botanical vogue of any period covered by the range of possible examiners, and consequently the syllabus is liable to overcrowding. It has been assumed, more perhaps for convenience than from conviction, that fungi are derived from algae and are therefore degenerate, hardly meriting the name of plant. Their physiology, moreover, is thought to be of little or no interest because of their lack of chlorophyll.

Berkeley ploughed a lonely furrow. It is interesting to speculate on the possible effect on British Botany if J. S. Henslow's wish that he should succeed him as Professor of Botany at Cambridge, had materialized. He was willing, though in the end he did not make serious efforts to obtain the chair, because he was advised that Babington's claims would doubtless be considered more favourably by his fellow residents. Berkeley was then 58, but regarded himself sufficiently young seven years later, to wish to accede to the suggestion that he should be a candidate for the Sherardian Professorship of Botany at Oxford, in succession to Daubeny.

As it was, Berkeley's influence was not on University students, but on amateurs, and they were not sufficiently trained to be able to follow the more abstruse and philosophical ideas which, under his guidance, might well have given abundant results; for the most part they restricted themselves to adding records to our fungus flora. C. E. Broome worked most closely with him, collaborating for over forty years, but others, less intimate, owed their introduction to the subject to him, directly or indirectly, through his writings.

The most prolific of these was M. C. Cooke. His *Handbook of British Fungi*, published in 1871, like many of his books, was chiefly compilation. His *Fungi, their Nature, Influence and Uses*, was a general account of fungi. It is often given as by Cooke and Berkeley, but the latter merely acted as editor, though he apparently drew up a plan for the work which he had originally undertaken. Cooke's *Mycographia*, 1875–9, a series of plates of Discomycetes, is not of very great merit and compares unfavourably with his *Illustrations of British Fungi*. This well-known and useful work has 1198 plates, but some of the plates were certainly not drawn from specimens, either fresh or dried. Cooke also began the cryptogamic periodical, *Grevillea*, produced first in monthly parts then quarterly, which served as a medium for the descriptions of fungi from 1872 to 1894, Massee editing the last two volumes. Unlike Berkeley, Cooke was able eventually to devote the whole of his time to mycology, for he was employed at Kew from 1880 to 1892. He was succeeded there by George Edward Massee.

One always feels that if Cooke had been less eager to see his name in

print and had devoted the time thus saved to serious work, he could have benefited mycology more. He had a wide knowledge of fungi but little critical faculty. His diatribes about such matters as heteroecism and the dual nature of lichens, show him as a die-hard; his constant obeisance to the authority of Berkeley and Fries was often just a smoke screen of defence. One strange breakaway was his strong advocacy of the introduction of the metric system to microscopic measurements.

W. W. Saunders and W. G. Smith published two parts of *Mycological Illustrations* with 48 plates, in 1871–2, but what might have been developed into a most valuable work, expired prematurely. Fortunately for British Mycology there were other contemporary authors. J. Stevenson's *British Fungi* (*Hymenomycetes*) in 1886 was a very useful and scholarly work based on *Monographia Hymenomycetum* and *Hymenomycetes Europeai*, where Fries had given the final results of a life-time's study. W. Phillips's *Manual of British Discomycetes*, 1887, showed how careful and original work could be accomplished by one whose livelihood was that of tailoring, and C. B. Plowright's outstanding *British Uredineae and Ustilagineae*, which appeared in 1889, gave the results of numerous experiments carried out by a busy general practitioner in a small suburban garden. Others were also adding to the superstructure, though they did not publish books. Amongst them should be mentioned Frederick Currey, a barrister, who wrote several valuable papers on Pyrenomycetes.

George Massee published a volume in 1891, with the title *British Fungi, Phycomycetes and Ustilagineae*—a most unsatisfactory production. His *British Fungus-Flora*, in four volumes, appeared 1892–5. Four volumes was the limit the publishers set, and only Basidiomycetes and Discomycetes were fully treated, together with a meagre account of Hyphomycetes. Like other writings of Massee, it is a mixture of sound fact and carelessness. Massee had as wide a knowledge of mycology as anyone of his period, but was quite unreliable. My feeling about his work is that one cannot accept his original statements on trust. All must be verified. They may be brilliant interpretations or they may be absurdities simulating boyish pranks in a superfluity of naughtiness.

Thus in the fifty years before the foundation of our Society we have Berkeley as the great influence until increasing age and failing health led gradually to comparative inactivity: but he was the acknowledged master to his death in 1889. Other mycologists came to the fore, but, with the exception of Plowright particularly and Currey to some extent, they were content with purely descriptive mycology.

Why was the British Mycological Society formed? What was the impetus? In 1868, Berkeley, with the object of bringing the subject properly before the public, induced Lady Dorothy Nevill and Mrs Lloyd Wynne to offer two special prizes of three guineas and two guineas for the best exhibit of edible fungi, staged at the General Meeting of the Royal Horticultural Society at South Kensington. Dr H. G. Bull of Hereford, in driving round to his country patients, had become interested in fungi and had begun to interest the Woolhope Naturalists' Field Club. On their behalf he staged an exhibit which was successful in obtaining the first prize.

Bull, a man of charm and energy, inspired the Club with his own enthusiasm and conceived the idea of holding an extra field meeting of the Club at Hereford for what he styled 'a Foray among the Funguses'. The first meeting in 1868 was a great success, and twenty-one sat down to dinner. The experiment was repeated annually in the first week of October with increasing success, and the Woolhope Foray became the recognized meeting place for mycologists. (The Woolhope Club was not the first to hold a mycological meeting: the Worcestershire Naturalists' Club had one in 1847.) Papers were read in the evenings and matters of mycological interest were discussed: thus, for example, Cooke's *Illustrations* was the outcome of a discussion about the need for an extended series of coloured plates. Many foreign mycologists attended. Bull was the driving force, and after his death in 1885, the Forays languished and ceased altogether in 1892.

There was an attempt to make Yorkshire the seat of an annual gathering of mycologists. The Yorkshire Naturalists' Union had held Forays in different parts of the county for several years and regularly from 1891. Though the Yorkshire Forays were, and still are, successful as county Forays, they did not attract as many mycologists from elsewhere as was hoped: M. C. Cooke and Carleton Rea attended regularly, as did G. Massee, who was a native of Scarborough. Yorkshire was more out of the way for most mycologists than was Herefordshire, the stalwarts (the Woolhopeans as they called themselves) were ageing and their numbers becoming depleted, and there were several annual Forays held in various parts of the country: those of the Essex Field Club were the best known.

As one reason for the Forays was to increase our knowledge of the fungus flora, there grew up a feeling that it would be better to hold meetings in different parts of the country rather than in any one county. A further consideration was the need for some vehicle of publication owing to the lapse of *Grevillea*. The active mover in the matter was Carleton Rea, who, having sounded several mycologists and got their support, pushed on with the project of a National Mycological Union, which was formally discussed at Huddersfield in 1895, and at the Selby meeting in September 1896, took form as the British Mycological Society, with Massee as first President, Charles Crossland, a well-known Yorkshire mycologist, a butcher by trade, as Treasurer, and Carleton Rea, a barrister, as Secretary: there were fifty members in the first published list (*Transactions* for the season 1896–7).

The first Foray was held at Sherwood Forest. Massee gave as his address 'Mycological progress during the past sixty years'. Only a résumé was published, which deals principally with the developmental studies of Berkeley, L. R. Tulasne, de Bary and Brefeld. Berkeley was primarily a systematist; the others in the years from 1840 onwards, by a series of brilliant researches on the morphology and life history of fungi, anatomical and then cultural, had revolutionized ideas on the group as a whole. Brefeld, indeed, introduced pure culture methods, using gelatine to solidify liquid media. Inoculation without previous sterilization, he said, was on a par with a man already soaked putting on a raincoat for protection against the last drops of rain. Nutrient gelatine as a solid medium was

introduced by Koch in 1881; in 1882 agar-agar replaced gelatine. Petri devised his famous dish in 1887.

The cytology of fungi had also begun to be studied. Their nuclei are usually very small and for a time they escaped observation; from 1879 their presence was recognized though precise details of mitosis were not described until 1895. From 1885 there was a series of researches on Phycomycetes, of which those of Harold Wager were amongst the first. Conjugate division of nuclei was first found in Uredineae in 1895, and the alternation of uninucleate and binucleate cells was described by P. Sappin-Trouffy in 1896. P. A. Dangeard, who directed Sappin-Trouffy's work, was one of the early investigators, and R. A. Harper had published his first papers; the two were about to take up the old controversy—Brefeld versus de Bary—which later had a side-show in this country between M. Hartog and A. H. Trow, over sexuality in Saprolegniaceae.

Thus fifty years ago the cytology in fungi was much in evidence. Plant pathology also showed signs of development. The outstanding British investigator with an outlook which has had world-wide recognition, was H. Marshall Ward. He was sent out to Ceylon to investigate the coffee scourge, *Hemileia vastatrix*, and in 1885 produced a masterly report. An epoch-making research was that on *A Lily Disease*. He was appointed Professor of Botany at Cambridge in 1895. Massee ended his Address with the words 'Now that Professor Marshall Ward is located at Cambridge, the growth of a British School of Mycology is simply a matter of time.' Unfortunately Ward barely survived the building of the new Botany School, dying in 1906, at the early age of 52. I have always regarded this as a tragedy for British Mycology. He was twice President of our Society, 1900 and 1901. Though admitting an inability to emulate the confidence of systematists, he confessed to a profound admiration of their powers of close and astute observation and retentive memories. I received my first botanical lectures from Marshall Ward, and well remember the thrill of his philosophical comments. Unfortunately, he was unable to give the half dozen lectures on fungi in the elementary course because of ill health, and I doubt if I ever heard him say more about fungi than that they were parasites and saprophytes. With an ever increasing number of students and adequate accommodation, he would have established a real centre for the study of mycology, which would have influenced British Botany and kept it from its mistake in underestimating the importance of fungi.

The widespread occurrence of mycorrhiza was known, but the work in this country was restricted to odd descriptions of the laboratory exercise type. Various fermentations had been studied, but principally those of yeasts. The work of van Teighem on the production of gallic acid by *Aspergillus niger* in 1867, and that of Wehmer on citric acid formation by *Penicillium* (*Citromyces*) spp. in 1892, were not given much attention. The general belief then, and indeed until very recently, was that the physiology of fungi was without interest, as it was chiefly a breaking down of carbohydrates to carbon dioxide.

Plant pathology was represented officially by Massee as part of his duties

at Kew: unofficially it hardly existed if we except the work which was beginning at Cambridge.

It would need a series of essays to trace the lines of the different developments of the study, which have taken place both here and abroad during the past fifty years. My attitude towards them is that it behoves a taxonomist to learn as much as possible of what is going on, for in his search for criteria to delimit and define his units of classification and to arrange them in what is called a natural system, he needs facts of all kinds. Odd clues can be gained in all sorts of ways. The end may be unattainable, but we travel hopefully. Taxonomy must lag a little behind the general advance, but it will only be by making full use of taxonomy that some advances can be made. There is a mutual dependence that is often lost sight of in the smoke of strife. So far as mycology in this country is concerned, however, we seem to have buried our hatchets in truly appropriate places—rather than in the places occasionally a little tempting—and it is now as rare for blame to be put on modern taxonomy for views expressed a century ago, as it is say for modern physiology to be similarly pilloried. This gives grounds for optimism. The taxonomist may well adopt the spirit and the words used by our national fighting optimist: 'Give us the tools and we will finish the job.'

In discussing the problems of taxonomy its two main objects should be kept in mind. The one is the identification of organisms, the second is their arrangement in a system. The two are linked by the necessity of determining the limits of the various categories—genera, species, variety and so on—and of a satisfactory nomenclature. About the necessity of precise identification there can be no question, but a few remarks on classification seem called for. There is no more helpful sign in modern mycology than the demoding of the so-called phylogenetic trees so popular for many years. In my undergraduate days they were set out in full array with all the arrogance of ignorance. Not only genera, but present-day species were given as the progenitors of smaller or larger groups. It did not seem to be realized that this implied evolution of a special kind in that in its more precise forms it postulated that all fungi have living ancestors. The best that can be said of such detailed phylogenetic trees is that they serve as useful mnemonics, providing branches on which the student can hang facts, but his right hand should know what his left hand does, or he will fail to realize the sinister implications. There can be nothing but good from general phylogenetic conceptions such as we shall doubtless have brought to our notice on Friday afternoon, but much detailed investigation requires to be carried out before we need worry about intensive philosophic conceptions: the details can be used to much better purpose for the gradual evolution of a reasoned classification. It seems to be too readily assumed that the doctrine of evolution is the basis on which classification builds, whereas in practice it is rather the reverse. Much could be said about this misconception and of the errors of reasoning into which it leads. Even without any theory, taxonomy would proceed as it always has done, with the aim of classifying organisms in the most convenient manner, which is to place together obviously related genera, species and other groupings. Theory may explain the similarities even if it be *quoddam supernaterale*, as Fries held. The process is

well seen in recent work on the Agaricaceae with the splitting off of a multiplicity of new genera and the resulting permutations and combinations. Incidentally, some of the splitting provides evidence of the distortion in classification brought about by overemphasis on a single character even if this is one needing a microscope for its elucidation.

On the whole it must be admitted that the present classification of fungi falls far short of a reasonable arrangement except for purposes of identification.

We have sketched the general position up to 1896, but without mentioning a work which had begun to have a very great effect. This was P. A. Saccardo's *Sylloge Fungorum*. The influence of this work on systematic mycology has been enormous. Here was an attempt at gathering together all known descriptions on a standard pattern. As Saccardo was a competent systematist as well as a compiler and bibliographer, he used his own arrangement and followed his own opinions about synonyms. The value of this work to mycologists is even greater than the *Index Kewensis*, originally subsidized by Charles Darwin, is to phanerogamists, for the latter does not give descriptions, but merely the authority and reference, and the earlier volumes even omitted the date. The first two volumes, devoted to Pyrenomycetes, were published in 1882–3; vol. III, Sphaeropsideae, 1884; vol. IV, Hyphomycetes, 1886; vols. V and VI, Hymenomycetes, 1887–8; vol. VII, Gasteromycetes, Phycomycetes, Rusts and Smuts, 1888; and vol. VIII, Discomycetes, 1889. Following this were a series of supplementary volumes keeping the work up to date; but also including a volume on Synonyms, one an Index of Species, two on Host Indexes, and two listing Illustrations.

Its very completeness was in some ways disadvantageous, for there was a tendency to use the work as a bible rather than what it really is, a most convenient index and commentary. Saccardo died in 1920, when vol. XXIII had been issued. The work was continued by his son-in-law, A. Trotter, but only two volumes were published, the last in 1931, including the additions up to 1917/8. The number of copies issued was very limited, but a facsimile reprint of the twenty-five volumes was published in America a short time ago.

With the spread of the study of mycology, many institutions and individuals were in need of this work. Now that they are able to obtain it at what must be considered a reasonable price, it will doubtless become the *vade-mecum* of a widening circle of mycologists.

In view of the probability of many foreign visitors being present to-day, I communicated with Prof. Trotter about the future of the venture. Having used *Saccardo* all my mycological life, I have my own ideas about its imperfections, so I feel fully entitled to speak about its value. It cannot be stressed too strongly that an effort should be made, international if need be, to ensure the appearance of additional supplementary volumes, bringing the descriptions of species and genera up to date. If something is not soon done we shall be faced with a situation resembling that prior to 1882. Then the trouble was that numerous descriptions had been overlooked because of their location in out-of-the-way books and periodicals. It is, if anything, worse now, for few, if any, are in the position of being able to look through all the periodicals which may contain accounts of new

species. Consequently, there are many chances of making synonyms and adding to the confusion.

In the new volumes all proposed new genera should be given, and all generic names, valid and invalid, should be listed with a view to the prevention of homonyms. The data of the original diagnoses should be given; anything new should be clearly indicated and not interpolated with no indication of its source.

Incidentally, a point in the International Rules of Nomenclature arises. A strict interpretation of Rule 38 (concerning the necessity for Latin diagnoses) would mean that after 1932 a published description does not become valid until it is Latinized, and thus if translated would date from its appearance in *Saccardo*. Meanwhile, it would be useful to have a list of the genera and species which have been described since the last volume of *Saccardo*, giving full references. A volume giving all generic names which have ever been proposed, with their original source, would eliminate the chief cause of perpetrating homonyms.

Prof. Trotter informs me that he has material for two, probably three volumes. The *Sylloge* is the property of Saccardo's family, and at present there are insufficient funds for publication. Money for this previously came from sales, but unfortunately the American reprint (for which no copyright payment was required because of the Alien Property Act) has interfered with these. Moreover, Prof. G. B. Traverso, through ill-health, is no longer able to collaborate.

I am hopeful that some scheme may be devised to ensure that this essential work shall proceed.

But this is very much now, what of then? If anyone had been asked in 1896 where was the greatest bottle-neck in Basidiomycetes, he would probably have indicated the resupinate fungi. The old descriptions based on hand and eye characters gave little certainty of identification, except where there was some outstanding character of colour, surface or margin. So soon, however, as they were systematically subjected to intensive microscopical examination, they proved amenable in a remarkable manner, the chief obstacle to ready apprehension being their multitude. Those who have contributed to this understanding are many, but credit for initiating the methods of investigation must be given to Bresadola and Massee—though, strange to say, Massee's *Monograph on the Thelephoreae* was amongst his most careless work. In former days, it was often assumed that these apparently formless and structureless fungi were probably dependent stages of more highly organized species, but it is now generally agreed that they are entities. I think, however, that we shall have to wait for culture studies before we can be absolutely certain about each and every one of them. So far, very few have been cultured. Moreover, occasionally we have an interesting polymorphism as in *Hypochnus fumosus*, and one knows from experience that where such a phenomenon is noted it is unusual for it to be isolated. Occasionally, in cultures, as Kniep found in *Armillaria mellea*, basidia with spores may occur on the mycelium without a fruit-body. I remember many years ago finding a *Sebacina*-like growth on a pine stump with basidia in abundance. It was strangely familiar, but I could

make nothing of it. I passed the stump practically every day and noticed that *Sparassis crispa* developed with identical basidia and spores. I do not suggest that these are more than anomalous.

The resupinate fungi as a whole may to many seem to have yielded their systematic harvest, leaving only the gleanings to be garnered. This is probably true if the methods of investigation remain stationary, but a closer study of basidial and other structural characters is giving interesting results. The time seems ripe for culture studies, which, if they do no more, will at least make our knowledge more precise.

Our mature mycologist, so despairing of resupinate fungi, would doubtless have maintained that the remaining Basidiomycetes were in good trim. The illustrious Fries had produced his classic, *Hymenomycetes Europaei*, in 1874. One hears so many books referred to as classics these days that I must not leave it at that. The work was fundamental, erudite, even inspired. It bears comparison with the *Species Plantarum* of that other great Swede, Carl Linné (Linnaeus), but the latter owed much of its earlier importance to its introduction of the binary system of nomenclature and of its present importance to its being the starting-point chosen for Botanical Nomenclature—Phanerogams, Algae and Lichens. Fries's volume, became as authoritative and exacting as Holy Writ. Only one shortcoming was gradually attributed to it, and that was the absence of spore measurements. In his introduction, written on his 80th birthday, he says that he had always been content to investigate the colour of spores and had not troubled about their size, though that was doubtless of importance.

He had laboured all his life at the larger fungi—his first paper was dated 1815—and having been the recognized authority on fungi since the appearance of his *Systema Mycologicum*, 1821–32, he was not prepared to attempt the putting of new wine into old bottles. The French mycologist, L. Quélet, first realized the great importance of spore measurements, but he gave only the length and the shape. Britzelmeyer provided wine without the bottles, placing such stress on spores that his descriptions are often nothing more than their dimensions and his plates are often such caricatures that it is as difficult to make out his new species as it is to find their names in the index. In spite of this there was more and more insistence on the value of spore measurement. Spore measurements taken from sporophores from widely separated regions correspond in a remarkable degree; on the other hand, there are discrepancies, some of which can be explained by the fact that the basidia are 2-spored or 4-spored, but some observers have found differences which cannot be accounted for thus. The real facts would be interesting, but at present we do not possess them. No value can be placed on measurements which are not made with some degree of physical exactness, and which are not presented in modal form. There can be no doubt that the size of the spores of a species is a valuable diagnostic character; the shape is at least as important, i.e. in three dimensions. The variation, however, usually seems to be too great for the minute differences sometimes recorded to be of any significance in the form they are given.

It is easy with the appropriate apparatus to be exceedingly nice in these

matters, and such nicety is seemly where it is warranted. To give a measurement in microns to three places of decimals even when it is put forward merely as an average, may be mathematically correct, but it is mycologically absurd.

It must be borne in mind that some of those who most strongly advocated the absolute specificity of spore dimensions within the narrowest limits, were often using faulty methods or faulty instruments. That the latter was so is seen from the fact that Boudier, one of the most careful of all mycologists, is reported to have used a faulty micrometer scale, so that all his measurements are 10% too large.* I never saw W. B. Grove measure a spore in the ordinary sense, but he judged them, and admittedly did so remarkably well. His method was one in vogue in the eighties. He describes this easy method in 1884, in *A Synopsis of the Bacteria and Yeast Fungi*—'Place your microscope in such a position that the image projected upon a piece of white paper is magnified 254 times: this can easily be done by a quarter-inch objective with the use of the draw-tube, or by placing the paper at a greater distance than ten inches from the eye-piece. Let this position be marked, so that the microscope can be placed in it again at any time. Now copy on the paper, from a scale, an inch divided into ten parts, and with a fine pen subdivide each tenth into five equal parts. Then the value of each of these subdivisions will be 2μ, and of the whole tenth of an inch, 10μ.' At one period there was controversy about the value of spore measurements; some thought these of little if any importance, basing their opinions on the measurements assigned by different authors to the same species, whereas others held that slight differences had specific significance. Both views postulated the exactness of all measurements, old and new. The point of these remarks is not to criticize the old work, but to stress that it is misleading to use the measurements there given, for purposes for which the data are unsuitable. A further warning: the spore measurements given in *Saccardo* are usually those of the original description; recrudescence does not confer exactness upon them.

The difficulty in interpreting spore measurements is that one cannot always judge what they signify. Thus to say that a spore measures $5–10 \times 5–4\mu$ may mean that a large number of measurements were made and that all fall within the given range; or that a rough fishing out of the largest and smallest spore gave the maximum and minimum. The practice should be followed of stating not only how the spores were measured, but also the method adopted of assessing the measurements.

Following the addition of spore measurements to the descriptions, attention began to be paid to other structures that can be measured. It was natural that basidia should be examined, for they bear the spores and were for a long time described as the fruit-bodies. The significance of basidial shape and structure had been realized from the work of Tulasne on Tremellineae and Dacryomycetineae in 1853, and in this broader sense was used for defining groups which formerly had been based on external

* M. Le Gal (*Rev. Myc.* II, 151 (1937)), however, regards the majority of Boudier's measurements as correct 'et lorsqu'il arrive que des mensurations ne sont pas exactes, celles-ci sont plus souvent *inférieures* que supérieures à la réalité'.

morphology and consistency. A good example of this was Currey's demonstration in 1861 that *Tremellodon gelatinosum*, in spite of its spines, should be grouped in Tremellineae because of its longitudinally cruciate basidia. It is generally conceded that the structure of the basidium is of phylogenetic significance, but there has not been any marked tendency to regard its general shape and size as specifically important, though many authors note them. If the same attention were given to their precise description as with spores, we should doubtless find that basidia are not so irrational as is frequently assumed. Greater care is necessary, however, than with spores, for it is the mature basidium that is in question, not young and not collapsed structures.

More attention has been given to cystidia. Lévéille gave these polymorphic structures their name, but they were described by most of the others who carried out those simple exercises, which, a little more than a century ago, revolutionized ideas on mycological taxonomy by showing that the spores were not borne in asci, but on basidia in what subsequently and consequently were called Basidiomycetes. Phoebus in particular dealt with them, but no effort was made to use them for diagnostic purposes until about sixty years ago: now they receive more than their proper share of attention. By this I mean that other parts of the fruit-body are almost always neglected as offering no differential criteria. But this is merely because the data obtained from certain characters are usually sufficient for identification and assuredly not that other characters are of no systematic worth. It should be clearly understood that if a restricted range of criteria is used for discriminating species then the definition of the species is circumscribed. This may usually be of no practical importance, but it is only by extending the range that we can approach certainty. It is more satisfactory to base judgment on definite microscopical differences in pellicle, trama, hymenium, stipe, annulus, and so on, than to place undue reliance on chemical tests. The modern tendency to use gross chemical manipulations to distinguish between species, particularly in the genus *Russula*, errs in using the tests both for definition and for recognition. If a given species is said to be the only one producing a certain reaction, then every specimen encountered which gives the reaction would inevitably be regarded as belonging to that species. Such chemical tests should be carried out with pure solutions and the result (or results) regarded as merely additional criteria and not as absolute.

It is often overlooked that Fries's system is entirely artificial and no manner of tinkering with it will make it anything else. Certain genera are obviously natural; others are gross conglomerations with groups of species clearly related but having no real structural similarity with other groups in the genus, but merely some semblance apparent only through the stressing of the equivalence of structures no matter what their origin. *Lepiota* is a good example of such a genus and the fact that species with green, pink and brownish spores occur within it is an indication of the inordinate importance which has been placed on spore colour in basing major divisions upon it. Fries's *Hymenomycetes Europaei* is a classic in the true sense, but this does not mean that it is more than a masterpiece of its period. The

time has long passed when any advantage can be gained by bolstering it up with characteristic species and relegating those that do not fit in as exceptions, with the distorted idea that exceptions prove the rule, and can be safely disregarded: it is the exceptions that should be treasured and studied, for it is from them that true ideas will be gained.

What we are most in need of at present are comparative developmental studies. Structures are not necessarily the same because the final results are similar, any more than the fact that we are here to-day postulates the method of arrival or our original starting-point. Doubtless we shall hear further of such matters at a later session.

The discovery of heterothallism in Basidiomycetes in 1918 added a new line of approach to several problems. So far the results obtained have not influenced taxonomy in its narrower sense, though a steady accumulation of facts should provide a wider basis on which to base theories and conclusions.

Perhaps cytology can best be mentioned here. Judging from text-books the opinion might be gained that this was the most important, indeed the only, aspect of mycology worth while. Fifty years ago cytological methods were beginning to be applied to mycology and provided strange and interesting results. Within ten years there was a spate of research, and it began to appear that the proper place for a fungus was in a pickle bottle previous to wax immersion. Sex in a cytological, even a psychological sense, was the vogue. For some reason cytology has passed out of fashion amongst mycologists. This is, one hopes, merely a temporary phase, for obviously there is here a fertile field. It is essential, however, that cytology in mycology should be servant and not master. It is the cytology of fungi which will provide results which are of value to mycology and not comparative cytology, for there fungi seem to fail in providing nuclei of sufficient size to warrant much attention in the unravelling of structural minutae. Combined with studies of development and sex, cytological investigations of even the much neglected Hymenomycetes provide abundant scope for profitable research.

It is only in recent years that plant pathology has become recognized by investigators as a discipline distinct from mycology, but there is still confusion amongst botanists generally, and, one may add, amongst administrators. There followed a tendency even to regard plant pathology as bearing little if any relation to general mycology. This point of view is happily now generally thought to be one which gives a false perspective. There is much in phytopathology which in no way concerns mycology: there is much that is pure mycology. Mycology is the study of fungi from every point of view. Their peculiar physiology imposes on them a multitude of activities: no matter how strange these activities they remain fungi, and it is by their manifestations we know them. Fungi being the proper study of the mycologist we cannot conceive of a Verboten because a family, a genus, a species, or a variety should have adopted a habit of parasitism on a cultivated crop; for apparently it is only the host of economic importance that is in question. This is a point of view that has been more or less generally adopted by British plant pathologists. I believe it will

continue to prove fruitful both to mycology and to plant pathology, for there cannot be any antagonism between the two. It would certainly be beneficial to mycology and probably to plant pathology, if more attention were paid to fungal diseases of native plants which have received no domestic pampering. From the literature it would appear that plants in the wild are as free from disease as certain theoretical sociologists assume that primitive man was before he paid the price of civilization. Possibly, by extending his researches to non-cultivated plants affected by similar fungi to those which particularly interest him, he might find profitable clues to help in theorizing about disease and immunity. Certainly the increased knowledge so gained would be invaluable to the taxonomist.

As things are developing I am sure that eventually we shall look among the plant pathologists for specialists in various groups. The wheel will have turned full circle.

Time necessitates that I should omit any detailed remarks about other groups. In all there has been a gradual but steady progress in precision of diagnoses. Rusts and smuts were in a reasonable state fifty years ago, and we know probably more about these groups than about any other. Heteroecism was well established at the time Plowright's book was published, and the concept of biological races announced by Erikson in 1893, proved, in the hands of the phytopathologists, one of the most fruitful of ideas and was gradually extended to other groups, and even to saprophytes, though here, as there is no selective host plant, we speak of strains. Smuts, though not so thoroughly studied as rusts, have provided evidence for the occurrence of hybrids, a matter not only of phytopathological interest, but also affecting taxonomic concepts.

The Pyrenomycetes are recovering from their setting in order by Saccardo, and the undue insistence on the value of spore difference—a single character criterion which divorces what structurally are obviously closely related genera. Boudier's insistence in 1885 on the essential difference in the method by which the ascus opens in Discomycetes and his reclassification made little impression at first, but with the years has gained wide acceptance. The foundation appears sound and greater emphasis given to anatomical structure and spore ornamentation has changed the group in a remarkable manner during the past half century, though the labourers in this field have been few.

Phycomycetes were favourite laboratory fungi in 1896, and hold their place for obvious reasons. Blakeslee's discovery of homo- and heterothallism in the Mucoraceae in 1904, was an epoch-making discovery both in science and in the elementary laboratory. In recent years in this country and elsewhere, there has been a revival of interest in aquatic fungi of all kinds, and we are rapidly gaining knowledge about their taxonomy, their ecology and their distribution.

Of the Fungi Imperfecti matters are much as they were fifty years ago in Sphaeropsideae, except that cultural studies have made for greater certainty in many ways.

In Hyphomycetes progress has been made along the lines advocated by Vuillemin in 1910, by placing importance on the true nature of the spores

judged by the manner in which they are formed. Moreover, it is in this group that most advance has been achieved in the chemistry of fungal products. The enormous success of penicillin as a therapeutic agent during the war has started a flood of research which will have its influence on the study of the organisms themselves. So far, though fungi may be harmful, their study has been free from all suggestions of anything but good—truly 'scientia amabilis' as Linnaeus termed botany. But with the threat of the use of biological warfare worse than the chemical and the physical of 1914–18 and 1939–45, it is not unthinkable that moulds may play a part, for the substances built up by them can hardly continue to be properly classed as 'harmless' or 'beneficial'. Further knowledge will add to the categories; indeed there are hints from America that this has already occurred and there are suggestions that laboratories have been erected elsewhere to investigate the possible production of such substances. If this be so it will add to mycological knowledge as well as chemical, assuming that the results are published. Otherwise, however, it can be no concern of mycologists, as such, though I doubt if anyone here is so completely absorbed in his special branch of science that he would remain complacent if he contemplated the possibility of knowledge being kept secret for such a purpose.

My remarks may seem to have been a little disconnected as they have had to be subjected to a time limit, and the scope of mycology is immense. Looking at the subject as it is now and comparing it with what it was fifty years ago, even as I knew it fourteen years later, I have a feeling of satisfaction at its growth and development. Mycology is in many ways a difficult study, but it is one that offers opportunities for original observations to those who have been trained in other branches of botany, even in other branches of science. Fungi are living and lively organisms—occasionally perhaps too lively—but a certain liveliness adds to the charm of living. They offer abundant problems calling for combined operations in their solution, and the germ of my theme is that taxonomy should seek its solutions from the answers to these problems. Moreover, they offer an unrivalled field to the amateur. Amateurs have played a prominent role in the mycology of this country, and in the history of the British Mycological Society. The technique for much of the study is very simple and the apparatus needed trivial. Thus, to me, one of the chief attractions of the important and fundamental researches carried out by A. H. R. Buller, whom most of us knew and whose recent loss we much deplore, is that they could almost all have been carried out with the simple equipment available to an amateur. Probably no other subject is in the position at present of offering prizes at so little financial outlay.

With the growth of our Society we have always endeavoured to ensure that the amateur has his true place, and this has doubtless had its effect on the preservation of the club spirit, which is one of the abiding charms of its gatherings.

Looking wider afield, what I feel is most needed in botany generally is an extension of the broad-minded outlook which is one of the most stimulating of mycology's many sane attributes. It is high time that the

foolish sectional interests in botany should be relegated to the limbo of lost causes. Mycology has suffered generally and taxonomy in particular, from lack of recognition. But the subject itself is living, and, in the words in gold letters over the choir of St Paul's—contained in the epitaph to Wren—*Si monumentum requiris circumspice.* This epitaph was in the depths of the vaults: it is now on high.

Mycology is the Cinderella of Botany and has suffered the disadvantages of step-sisterhood. The rest of the family at one time or another has received recognition, and occasionally with little warrant except that of importunity. But Cinderella is now fully attired for the Ball. Indeed the carriage is waiting. She has all the characteristics which usually attract in that she has developed in a comely manner and has charms of which her devotees are aware, and—she can bring her quiver full of rations for the general good. May those who have served her faithfully benefit for their devotion, and in particular may the British Mycological Society grow from strength to strength in the spirit in which it was founded, and with the single-hearted purpose and broad outlook with which it has continued.

MEDICAL MYCOLOGY

By C. W. EMMONS
Principal Mycologist, National Institute of Health, U.S. Public Health Service, Bethesda, Md.

An etiological relationship between fungi and certain human diseases was observed more than 100 years ago. In 1839 Schoenlein described the fungus which causes favus, and Lagenbeck the fungus which causes thrush. These and other early contributions to medical mycology were largely clinical descriptions of the diseases and there was little mycological work. A second period during which more attention was given to the fungi associated with human mycoses has occupied the past fifty years. British mycologists have made notable contributions during both periods.

Mycology has lost a valuable opportunity by failing to study more systematically those fungi which cause human disease. Medicine as well as mycology has lost, because the trained mycologist, collaborating with the clinician, can make an effective contribution to the systematic study of medical mycology. Reasons for the neglect of this field are probably related in part to the phenomenal impetus given to bacteriology by Pasteur, Lister and Koch; to the fact that bacterial diseases of man are more frequent than those caused by fungi; and to lack of contact between the mycologist and the hospital diagnostic laboratory. Financial support of medical mycology is still rare and uncertain, but there is now an increasing tendency to recognize the importance of fungi among the other etiological agents of human disease, and to enlist the aid of the mycologist in their study. Prior to this recently awakened interest it is probable that many mycologists had never seen some of the pathogenic fungi which are known only from man. They knew others from soil and vegetation, for some of the fungus pathogens of man appear to be primarily saprophytes with a natural habitat unrelated to a mammalian host. The mycologist should be informed about the fortuitous parasitic relationship which these erstwhile saprophytic fungi occasionally establish.

Unlike plant diseases, most human diseases are caused by bacteria or viruses. The mycoses (with few exceptions such as athlete's foot and other types of ringworm) are unknown to the layman and have no common names. They are relatively unknown to medicine as well. Even mycoses which are world-wide in distribution are sporadic and rare in occurrence according to our present knowledge of them. Yet, when we compare the mycoses with some of the better known human diseases, they assume a greater importance. In *Vital Statistics of the United States for* 1942, 1,385,187 deaths were reported. Of these 359 were attributed to fungi. This is less than 0·03 % of the total, but in this registration area it is nearly twice as many as the total of known deaths due to paratyphoid fever, undulant fever, smallpox, rabies, leprosy, plague, cholera, yellow fever,

and relapsing fever together; it is greater than the number due to all the typhus-like diseases together; and it is more than half the number due to either typhoid, tetanus, or poliomyelitis. For fairness in comparison it should be pointed out that many of these better known diseases rarely cause death, because of effective prophylactic and control measures. On the other hand, some of the mycoses (particularly the dermatophytoses and moniliases) are not fatal diseases, although common and frequently annoying. It must be further pointed out that there are mild forms of some of the generalized fungus infections in which a correct diagnosis is not always made and, consequently, the true incidence of the mycoses is not known. This may be illustrated by the following example.

Coccidioidomycosis is a mycosis once thought to be invariably fatal, but now known to be mild and self-limited in the great majority of cases. This mycosis will be discussed later in another connexion, but its history may be traced now in order to illustrate the point in hand. The disease was first observed in Argentina, but relatively few attacks have been reported from that area. The second and third cases were reported in 1894 by Rixford and Gilchrist from California, and thereafter many additional cases were seen, particularly in the San Joaquin Valley of southern California. The disease, which was named coccidioidal granuloma, was highly fatal and no specific treatment was known. It was made reportable in that State and several hundred cases were accumulated during the ensuing thirty-five years. During this period, as the mycosis became better known and laboratory methods of diagnosis became more widely used, a few milder cases were seen, and the recognition of self-limited cases reduced the accepted mortality rate from nearly 100 to about 40 %.

In 1937 a new era in knowledge of the disease was opened through the studies made by Gifford and by Dickson of an acute respiratory disease of unknown etiology present in the same area. This disease had been known for many years in the San Joaquin Valley and was commonly known to the layman as 'Valley fever' or 'desert rheumatism'. Laboratory investigations of Valley fever made in 1937 revealed that it had the same etiology as coccidioidal granuloma, and the subsequent painstaking investigations of Dickson and of Smith had demonstrated that there is one disease, that it begins in nearly all cases as a respiratory infection following inhalation of air-borne spores, that it usually runs an acute but self-limited course without sequelae, and that only in rare instances, perhaps one in 5000, the disease becomes systemic and then presents the classical syndrome of coccidioidal granuloma, with consequent grave prognosis. The relationship between these two phases of coccidioidomycosis has been further elucidated by Smith and his associates during the war, in personnel of the armed forces of the United States who were trained within the endemic area of this mycosis. A skin-testing antigen, coccidioidin, which has a good degree of specificity, has been used in these studies. It was found that some men who did not react to an intradermal injection of this material when they first entered the endemic area did react when retested a few months later, although no acute illness had occurred in the meantime; that others first became reactive after an acute respiratory episode of the Valley fever type;

and that the expected very small number developed disseminated coccidioidomycosis (coccidioidal granuloma).

The occurrence of a mild form of coccidioidomycosis is thus firmly established. Does such a relationship exist in other mycoses which now appear to be fatal and rare? It is a possibility which must be considered. We have some reason to suspect that there is a mild unrecognized form of histoplasmosis, and I shall mention this briefly as a second possible example of a mycosis which is rare and almost invariably fatal so far as proved cases are known, but which perhaps occurs also in a common and milder form. However, it must be understood that the evidence for the existence of an unrecognized benign form rests upon analogy with coccidioidomycosis and upon the very dubious evidence of a skin test which we know is not specific.

Since its discovery and description in 1906 by Darling, histoplasmosis has appeared rarely and sporadically throughout the world until at the present time something like 100 cases have been observed and most of them reported. Most have been observed in the eastern half of the United States, but there is no clear evidence concerning the actual geographical distribution. The significant facts for the present discussion are that almost all of these cases have been fatal and that there have been no recognized cases of transmission from person to person, although we reported last year the first known instance of histoplasmosis in brothers and transmission may have occurred here. The source of infection has not been determined in any case. Do the cases which have been diagnosed represent only the more severe cases in which recognition has been easier or in which by chance, laboratory examination has been made and the fungus observed? The wide occurrence of skin sensitivity to histoplasmin has been interpreted by some investigators as evidence to support an affirmative answer to the question. Histoplasmin is a sterile filtrate of a synthetic broth culture, which when injected intradermally in a sensitized individual produces within forty-eight hours a localized area of edema resembling a positive tuberculin test. Such a reaction occurs in laboratory animals infected with experimental histoplasmosis, and in many people who are not ill and have no history of an illness resembling histoplasmosis. In several proved human cases of histoplasmosis where the test has been used the patient has not reacted. The percentage of the general population reacting to the histoplasmin test differs greatly in different geographical areas and with age.

There seems to be some degree of positive correlation between sensitivity to histoplasmin and the presence of calcified pulmonary lesions, and it has been suggested therefore that there is a common benign form of histoplasmosis which produces lung lesions that heal and become calcified. The evidence for this view is weakened by the fact that the correlation is not complete, and by the observed cross-reactions of histoplasmin in other mycoses. Proof of the existence of mild forms of histoplasmosis, and of other mycoses which are now known only in grave forms, awaits actual demonstration of the fungi in the hypothecated new clinical forms. We have isolated *Histoplasma* once from a hilar lymph node in a child dying of another disease.

In reviewing the importance of the mycoses one can point out that some of the superficial fungus infections such as dermatophytosis and secondary moniliasis are extremely common and annoying; that the mycoses as a group are more frequent causes of death in the United States than many better known bacterial diseases; and that at least one (coccidioidomycosis) and perhaps others are more frequent in mild form than their recognition indicates. Until we know more about the diagnosis and clinical variations of some of the apparently rare mycoses we can only speculate upon their actual frequency.

The diagnosis of mycotic infections rests largely upon the laboratory. Even the superficial skin lesions caused by the ringworm fungi may resemble so closely certain dermatoses due to skin irritants or sensitizing chemicals that a demonstration of the fungus in epidermal scales by microscopic examination or its isolation in culture is necessary in order to make a differential diagnosis. In the systematic mycoses the demonstration of the fungus microscopically or by culture is even more important in establishing the diagnosis. Fortunately, in dermatophytosis, epidermal scales or infected hair stubs are plentiful and easily obtained, and the laboratory procedures are reasonably effective. Epidermal scales are readily cleared by warming in a drop of 10 % sodium hydroxide on a microscope slide. The fungus hyphae are much more resistant to digestion and can be easily observed by searching a field with the low-power lens. Likewise the isolation of the fungus in pure culture is not difficult. Epidermal scales are immersed for two to ten minutes in 70 % alcohol and then placed upon agar slants. The bacteria commonly present on skin are much more susceptible than the fungi and are killed by this exposure. Many media are suitable for cultivation of the pathogenic fungi, but traditionally some modification of Sabouraud's agar is commonly used. Difco neo-peptone 1 % and chemically pure dextrose 2 % is a convenient and excellent culture medium.

The deeper mycoses require slightly different procedures. *Sporotrichum* is easily isolated from the subcutaneous abscesses of sporotrichosis by withdrawing pus from an unopened and therefore uncontaminated abscess and spreading it upon agar. *Histoplasma* can be isolated from the circulating blood or from sternal bone marrow, and *Cryptococcus* can be obtained in pure culture from spinal fluid in cases of meningitis. In pulmonary mycoses and those with ulcerative skin and mucous membrane lesions, where secondary bacterial contamination is sure to occur, bacteriological methods of spreading and picking pure colonies can be used. Differential media are of assistance. Media with an acid reaction are commonly used, but acidity alone will not prevent the growth of the bacteria usually present in sputum and on the skin. Smith recommends treatment of sputum with 0·05 % copper sulphate and incorporation of copper sulphate in the medium in the isolation of *Coccidioides* from sputum. Penicillin, to which most fungi are very resistant, can be added to medium in amounts up to twenty units or more per millilitre.

The serological methods of diagnosis so useful in the bacterial diseases are not commonly used in mycoses. The production of antibodies by the fungi seems to be much more erratic. In American blastomycosis or

Gilchrist's disease, for example, Martin has reported that in some proved cases antibodies are not demonstrable. In coccidioidomycosis Smith has observed that the complement-fixing titer rises as the disease progresses from the acute respiratory phase to the chronic, disseminated, granulomatous phase. The early serological studies of Davis demonstrated the identity of American and French sporotrichosis. Agglutinins, precipitins and complement-fixing antibodies are produced in many mycotic infections, and it is to be hoped that improvement and standardization of techniques will increase the usefulness and dependability of these methods in the diagnosis of mycoses.

The geographical distribution of the human mycoses is world-wide or nearly so in many cases, but restricted to certain areas in a few. Dermatophytosis extends around the world, but the prevalent species of dermatophytes, and consequently the clinical types of lesion, vary to a considerable extent depending upon geographical areas. The reasons for the geographical localization of species may be climatic. *Trichophyton rubrum* is more prevalent in tropical and subtropical areas than in northern zones, for example. It may be dependent upon customs and living conditions of the population. Exposure to an animal host or reservoir is a determining factor in the case of farmers whose cattle may develop ringworm.

Actinomycosis caused by the anaerobe, *Actinomyces bovis*, occurs throughout the world. Sporotrichosis caused by a single species, *Sporotrichum schenckii* has been reported from the gold mines of South Africa to the plant nurseries of northern United States. Histoplasmosis is caused by a single species throughout the world.

In some cases a mycosis appears to have become naturalized in a new area after its accidental importation. Thus the striking dermatophytosis known as tokelau or tinea imbricata, which is native to certain South Pacific areas, was brought to Central America probably during slave days, and now occurs in localities there. During a visit to Guatemala I saw perfectly classical examples of this striking skin disease, in which partially adherent epidermal scales form concentric and confluent patterns over the entire body surface.

On the other hand, a few mycoses are restricted in distribution. Coccidioidomycosis is endemic in the arid south-west of the United States and a similar area in Argentina. The few cases seen elsewhere in the world can probably be explained by exposure during an earlier residence of the patient within the known endemic area of the mycosis, or by exposure to dusty packing materials, fruit, and other products imported from such areas. North American blastomycosis or Gilchrist's disease is another peculiarly North American disease, most often seen in the United States but occurring also in Canada. South American blastomycosis (paracoccidioidal granuloma) is primarily a Brazilian mycosis, but it occurs in other South American countries. Until we know more about the reservoirs of these diseases, or the natural habitats of the fungi which cause them, we shall find it hard to explain these geographical limitations to distribution.

Although I know that many of them are already familiar to you I propose to discuss briefly some of the more common mycoses and the fungi which

cause them. By far the commonest mycosis is dermatophytosis. In one or another of its clinical types it attacks man's integument from the sole of his foot to the crown of his head. Because it produces circular, spreading, scaly, itching areas on the glabrous skin and conspicuous bald spots on the scalp, and because the fungus is large and easily demonstrated in epidermal scales it is not surprising that some types of ringworm were among the first infections in which an etiology was proved. Favus has already been mentioned as an example. Other clinical types were also studied by the microscopist.

Sabouraud's classification of the dermatophytes was based primarily upon the spatial relationships of the fungus to the parasitized hair shaft and upon clinical characteristics which were in part determined by these relationships. Numerous attempts to substitute a mycological classification have been made. Well over 200 names of dermatophytes occur in dermatologic literature as a result of new combinations, renaming validly named species, and the indiscriminate splitting of species. Actually Sabouraud's classification resulted in a grouping of species which, with some exceptions, coincides with a natural division of the group. Unfortunately many of his species names are invalid.

The dermatophytes are an interrelated group of fungi with some morphological features in common, and the peculiar physiological adaptation to a parasitic existence on the cornified or keratinized tissues of man and animals. Large clavate to fusiform macroconidia are found in each of the three genera of dermatophytes, *Microsporum*, *Trichophyton* and *Epidermophyton*, although they are not produced by all species, and the shape is characteristic for each genus.

Ringworm of the scalp has been sporadic or has appeared in only small epidemics in the United States until within the past five years, when extensive epidemics have occurred in schools in many of our cities. In many cases these have severely taxed the treatment facilities of the communities involved. Their control has depended upon prophylactic measures in barbers' shops, schools, and theatres, and upon improved methods of topical treatment to supplement the standard treatment of X-ray epilation followed by application of a fungicidal ointment.

Sporotrichosis was first described from the United States, but it is of frequent occurrence in France and in many other parts of the world. The primary lesion is often an ulcer on the finger at the site of an injury such as a thorn or splinter puncture. In the tissues the fungus grows as a cigar-shaped cell which reproduces by budding. In culture *Sporotrichum schenckii* is a variable fungus and the many colour and colonial variants have been given individual names of very doubtful validity. Strains newly isolated are often almost black, but repeated subculture of such a strain usually gives rise to a colourless mutant. The conidia are smaller than those of most saprophytic species of *Sporotrichum*: they are borne at the tips, and along the sides of slender tapering conidiophores, and along the sides of undifferentiated hyphae.

Perhaps the most striking example of dimorphism in the fungus pathogens of man is offered by *Coccidioides immitis*. It reproduces in animal tissue

solely by progressive cleavage of the protoplasm in the manner so well known in the Phycomycetes. The section illustrated here was the first published demonstration of the nuclei of this fungus. The *Coccidioides* cell increases in size and develops into a sporangium which upon maturity ruptures, and the cycle is repeated. On agar *Coccidioides* is a rapidly growing mould in which more or less specialized hyphae bear chlamydospores. These are produced in very large numbers, are easily disseminated, and presumably constitute the infectious form of the fungus. When injected into an animal each chlamydospore is converted directly by growth in size and by progressive cleavage of the protoplasm into a sporangium.

American blastomycosis or Gilchrist's disease may present either localized skin lesions which progress slowly with ulceration and microabscess formation at the advancing raised border and heal with severe scarring in the older part of the lesion, or the infection may be generalized with pulmonary involvement, the most frequent manifestation. The fungus is found in the microabscesses as large thick-walled budding cells 8–15μ in diameter. When the infected pus is planted on agar the fungus grows as a Hyphomycete bearing spherical conidia on slender conidiophores. If a culture is injected into an experimental animal the fungus reverts at once to the budding phase.

Histoplasmosis is a fatal generalized infection which is unlike most of the pathogenic fungi in its predominately intracellular parasitism. The small, delicate budding cells of *Histoplasma capsulatum* are found in macrophages and rarely in polymorphonuclear leucocytes. The disease was discovered by Darling in Panama while searching for kala azar. The fungus was described from pathological sections only, under the impression that it was a protozoan similar to *Leishmania*. Its mycotic nature was subsequently recognized, but no case was diagnosed before death until 1934, when De Monbreun isolated the fungus in culture. The disease is of great interest now, because of the possible association already discussed, with non-tuberculous pulmonary calcification. When the fungus grows on agar it is a dry white mould resembling *Blastomyces dermatitidis*, but easily differentiated from it by production of large conidia having conspicuous finger-like appendages. Some strains can be maintained in the yeast-like phase in culture on blood agar incubated at 37° C. Histoplasmosis occurs in dogs and can be readily transmitted to most laboratory animals.

Chromoblastomycosis is a chronic granulomatous disease usually involving an extremity. The primary lesion is an ulcer or sometimes a papule which develops at the site of an injury such as is caused by a thorn or splinter. It fails to heal and extends slowly, forming warty or cauliflower-like lesions. The disease may have a duration of twenty-five years or more without extending beyond the foot or ankle. In most cases, however, satellite lesions develop as a result of autoinoculation by scratching, so that there are multiple lesions extending up along the lower leg, or along the arm when the primary lesion is on the hand. In the superficial scales of the lesion the fungus is present in the form of abortive hyphae and short mycelial elements. In the subcutaneous tissues the fungus elements

are almost spherical, or slightly elongated, and divided by cross walls into clusters of small cells which on superficial examination suggest clusters of budding cells. The fungus is dark olivaceous and the combination of a resemblance to blastomycosis and the pigmented character of the fungus was responsible for the name of the disease, chromoblastomycosis.

The fungi causing this mycosis are of especial interest to mycologists because of their close resemblance to familiar saprophytic fungi. One of two is commonly involved. The first of these, *Phialophora verrucosa*, was shown by Conant to be the same as *Cadophora americana*, which had been subsequently described as one cause of discoloration in lumber and wood pulp. The second has been known under a variety of names, the best known of which is *Hormodendrum pedrosoi*. The two types of sporulation exhibited by these fungi are quite distinct. However, Dr Carrion and I found, upon careful study of these fungi, that while one spore type predominates in each species, both types are actually present in each.

This conclusively demonstrates a close relationship between the two fungi and the expression of this close relationship has been attempted in various ways by different workers. It seems to me most logical and correct from the mycological viewpoint to make the two species cogeneric and to place them in the oldest genus, namely *Phialophora*, at the same time broadening the definition of this genus to accommodate the two types of sporulation. Both these fungi are present in soil or decaying vegetation, as demonstrated by initiation of infection after trauma, and the well-known occurrence of these fungi or closely related species in such substrates.

Aspergillosis is a pulmonary disease of man and birds. It is rare in the United States, but occasional human cases are reported, and it is found in turkeys, pigeons and occasionally in wild species of birds in captivity. In the parasitic phase contorted, septate hyphae are found in sputum and in pus and *Aspergillus*-type conidiophores are sometimes found in air spaces of the lungs. The pathogenic species is *A. fumigatus*, which is a familiar species. *A. niger* is the most frequent cause of aspergillosis of the ear canal.

There are a number of other fungi which are primarily saprophytes but which can on occasion attack man. In some cases at least, these fungi assume the parasitic habit only when introduced to the subcutaneous tissues through an injury.

Many of the problems of nomenclature which beset medical mycology have been mentioned or were apparent in the brief discussion of specific mycoses. Some of them are peculiar to medical mycology while others are the familiar ones of taxonomic mycology. There has perhaps been more than the usual number of selections of an invalid name for a fungus (e.g. *Torula* and *Blastomyces*); there has been an enormous number of transfers from genus to genus; a great many fungi have been repeatedly renamed by investigators who were ignorant of the earlier literature or who capriciously rejected a valid name in favour of a new one; and there have been too many exponents of the policy of splitting species on the basis of slight differences between strains. The variability of fungi is a fascinating subject, and if there were time I should like to discuss the several hundred strikingly different mutants which Dr Hollaender and I isolated from

a strain of *Trichophyton mentagrophytes* following ultra-violet irradiation of conidia. Conidia of a typical strain of that species were exposed in suspension in a quartz cell to monochromatic radiation of wave-length 2537 or 2650 A. for a time sufficient to kill 90–99% of the conidia. The surviving spores were plated out and individual colonies showing obvious variation from the type were isolated. The mutants induced were striking and permanent. Some of them resembled other recognized species of dermatophytes.

What is the natural habitat of these pathogenic fungi? They can be separated roughly into groups, in one of which the fungi seem to be normally associated with man, and another in which they appear to be in his environment and become pathogenic only by chance. The first group includes the dermatophytes which are highly specialized for successful parasitism and are transmitted from person to person. They are capable of saprophytic growth, but it is probable that in nature they compete successfully with other saprophytes only on debris of animal origin.

Candida albicans probably is present frequently on the surface of fruit, yet it is much better known as an almost constant inhabitant of the respiratory and gastro-intestinal tract of man. This yeast-like fungus is unquestionably pathogenic and is a cause of thrush, vaginitis, and certain types of eczematoid skin lesions. It is also associated with pulmonary disease, but the fact that it is almost ubiquitous in the upper respiratory tract makes an evaluation of its role in pulmonary disease difficult indeed. It is present in sputum as a secondary invader in almost all types of pulmonary disease, and although it may have no more etiological significance than the saprophytic bacteria which are also present in sputum, the healthy human body is undoubtedly one of the normal habitats of this fungus.

Actinomyces bovis, contrary to the oft repeated text-book statement that actinomycosis is contracted by chewing straws, has not been isolated from any source except the human or animal body. It can be isolated readily by appropriate methods from the surface of discoloured teeth, dental cavities, and tonsillar crypts in persons without actinomycosis. It is an almost constant inhabitant of the oral cavity but rarely causes disease.

Some of the other pathogens can be fairly definitely associated with a natural habitat in soil or decaying vegetation. *Aspergillus fumigatus* is such a fungus. *Nocardia asteroides*, or a very similar pathogenic species, has been isolated repeatedly from soil. Sporotrichosis is definitely associated with exposure to and injury by plant material and the pathogen has been isolated from sphagnum moss, plants and soil water. The species of *Phialophora*, which cause chromoblastomycosis, are soil or decaying wood inhabitants and injury plays the necessary role of permitting penetration of the skin. *Coccidioides immitis* has been isolated from the soil, but the relationship here may not be as simple as with the other fungi just mentioned.

In investigating the habitat of this fungus in the desert area of southern Arizona I trapped and examined several hundred wild rodents. *Coccidioides*, and a second related fungus (*Haplosporangium parvum*) which has not yet been found in human disease, were found in a high percentage of animals of some species. These fungi were found in pulmonary lesions in some 80%

of pocket mice and in a high percentage of kangaroo rats and ground squirrels. They were rarely found in the white-footed mouse which was equally common. *Coccidioides*, in the susceptible species, caused a chronic, slowly progressive mycosis which had a specific relationship to certain species of rodents, while other species with apparently the same exposure were rarely infected. The explanation which seemed best to fit the observed facts was that coccidioidomycosis is primarily a disease of rodents and that it is secondarily present in soil contaminated by infected rodents. This relationship requires further study. We still lack information on many problems of transmission and diagnosis of mycoses.

I have tried to point out in this discussion of medical mycology its relationship to general mycology and to medicine; the relative importance of mycoses which are more common than is generally realized; the outstanding characteristics of some of the more important mycoses; the peculiarities of the fungi of medical importance; and some of the unsolved problems involving the natural habitats of these pathogenic fungi and the transmission, diagnosis and treatment of the human diseases they cause.

SOME ASPECTS OF FUNGAL METABOLISM; WITH PARTICULAR REFERENCE TO THE PRODUCTION OF ANTIBIOTICS

By J. H. BIRKINSHAW
London School of Hygiene and Tropical Medicine

(With 7 Text-figures)

Until the last decade of the nineteenth century, few contributions were made to our knowledge of the metabolic products of the fungi. The botanist engaged in taxonomic studies was able in some cases to observe crystals in the tissues of the fungi he was examining, but his chemical knowledge and technique were not usually sufficient to attempt the identification of anything more difficult than calcium oxalate. The chemist, on the other hand, had usually little interest in fungi and he was certainly unable to undertake the isolation, identification and maintenance of pure cultures. Thus we find that even the few earlier records of biochemical investigations available are in some cases marred by the use of mixed cultures and incorrectly identified species. The name '*Penicillium glaucum*' covered a multitude of species, as it might be applied to almost any green *Penicillium*. In such cases it is of course quite impossible now to trace the species actually used.

In spite of, or perhaps I should say because of, the lack of knowledge obtained by actual experiment, the impression seems to have prevailed that the fungi, and in particular the microfungi, were mainly efficient transformers of carbohydrate material into carbon dioxide and water, thus performing the role of scavengers in the economy of nature. This slow combustion process was supposed to be accomplished with such efficiency that intermediate products capable of isolation were not formed in appreciable amount.

Real interest in the biochemistry of the fungi was first awakened in the nineties by the classical researches of Carl Wehmer, who combined in himself the functions of mycologist and chemist. Wehmer showed that *Aspergillus niger*, when grown on sugar solutions, was capable of producing oxalic acid which accumulated in the culture solution. He also showed that citric acid is a metabolic product of certain fungal species for which he created the genus *Citromyces*.

In 1917 a further notable advance was instituted by Currie, who, himself a chemist, had the great advantage in his earlier work of collaboration with Charles Thom, the eminent mycologist. Currie noticed that oxalic acid formation by *Aspergillus niger* did not run parallel with the acidity developed in the culture fluid but that there was a distinct lag, which on investigation proved to be due to citric acid. Citric acid is indeed produced from sugar in high yield by many of the microfungi. The main merit of Currie's discovery lay in the fact that he was able to develop the citric fermentation into a commercial process employing *A. niger* as agent for the production of citric acid from sugar. The process became firmly established and is now the major source of the world supply of citric acid. Wehmer had tried to

commercialize his discovery of the citric acid fermentation but failed. Currie's later success must be attributed to the fact that he employed a much more vigorous organism, a rapid grower, with higher optimum temperature and capable of withstanding a very low pH, so that sterilization problems became simplified.

These observations led to the acceptance of the view that in some instances at least the lower fungi are capable of producing a fermentation comparable in importance with the alcoholic fermentation due to yeast. They also indicated the strong desirability or even the necessity of adequate representation of both the mycological and biochemical aspects in work of this nature. The mycologist and the biochemist should work hand in hand, each contributing his share to the total result.

Such was the position when Prof. Raistrick and a small team of workers began the study of the metabolism of the microfungi about twenty-five years ago in the laboratories of Nobel's Explosives Company (now Imperial Chemical Industries) at Ardeer. The study was continued from 1929 at the London School of Hygiene and Tropical Medicine. In this work, in which I was privileged to participate, the initial aim was to obtain general information as to the metabolic products of the microfungi by a preliminary survey of the field.

It was evident that it would be impossible to investigate in detail in a reasonable space of time the chemical compounds formed by even a small proportion of the different known species of fungi. For this reason, instead of attempting the isolation and identification of the compounds formed by any species selected at random, it was decided to investigate quantitatively the types of compounds formed by each of a large number of fungi so as to obtain a logical basis for the choice of any particular fungus for later investigation. To this end a method involving the preparation of carbon balance sheets was evolved. The method used was capable of being applied to any organism which would grow on or in synthetic media.

The form of apparatus devised enabled the organism to be grown in a closed system equipped for aeration, so that all the volatile products and CO_2 generated by the metabolic processes were trapped and measured. At the termination of the incubation period the mycelium and culture fluid were analysed. In this way the carbon present in the following classes of compounds was determined:

(1) Carbon as volatile compounds: (*a*) CO_2, (*b*) volatile organic compounds.

(2) Carbon in organism.

(3) Carbon in culture solution: (*a*) residual glucose, (*b*) CO_2 in solution, (*c*) volatile acids, (*d*) non-volatile acids, (*e*) volatile neutral compounds, (*f*) synthetic compounds precipitated by colloidal iron, (*g*) unaccounted carbon.

A quantitative survey of the *types* of organic compounds produced by any particular organism was obtained. Those species which from this preliminary survey appeared to be of special interest were then further examined. In this way a number of new metabolic products were isolated.

On the mycological side every effort was made to ensure the authenticity

and purity of all cultures used. All fungi used were received from outside sources such as the various Type Culture Collections, or were isolated in the laboratory. They were carefully checked by a competent mycologist and single spore cultures made from them. In any doubtful cases the identification was further checked by submission of the cultures to an outside expert, and we are particularly grateful to Dr Charles Thom of the Microbiological Department of the U.S. Department of Agriculture for the large number of identifications which he undertook on our behalf.

Objection to this type of work has occasionally been raised. Although the identification of new metabolic products is no doubt very interesting, it is argued, they do not seem to throw much light on the mechanism of the metabolic processes which is the true aim of metabolic studies. To that argument I would reply that although no doubt the study of the inter-relationships and interconversion of these fungal products and elucidation of the processes by which they arise or disappear is the more distant aim which it is eventually hoped to attain, the intermediate object must be to discover the compounds whose relationships are to be studied. The present knowledge of the yeast fermentation process could not have progressed very far without the important discovery by Harden of the phosphorylation of glucose.

One interesting and somewhat unexpected outcome of the early work of Raistrick's group was the observation that when the fungi of a particular genus are classified according to their biochemical characteristics, as revealed by the type of product predominating, the scheme so presented may follow closely the classification based on morphological characteristics as used by mycologists. This is especially true of the Aspergilli, but less marked in the Penicillia. With species of *Fusarium*, on the other hand, there seems little hope of classification on these lines, since all species tested gave rise principally to alcohol and showed themselves biochemically allied to *Saccharomyces*. Although slight quantitative differences were observed these were not sufficient to permit of a biochemical classification.

The balance-sheet method, whilst of value in detecting major products of fermentation, is not a sufficiently delicate instrument to indicate products formed only in small yield, which may, nevertheless, be of considerable significance. We found it advisable to add to our armoury other sorting tests of a chemical and latterly of a biological nature. As an example of the chemical test, I would mention the ferric chloride reaction which reveals the presence of products of phenolic nature and those possessing a keto-enol group. The biological test which has lately come into prominence is of course the examination for antibacterial or antifungal activity.

As a result of the efforts of workers in laboratories in many different parts of the world a large number of fungal metabolic products have now been described and characterized. These comprise substances of many different chemical types, and present a striking picture of the multifarious activities of the fungi. It is not my intention to survey the whole of this wide field, but rather to select a few groups of chemically related fungal products in order to indicate the type of pattern which is gradually being revealed and which we hope will eventually enable a composite picture of fungal metabolism to be presented. In this selection I shall pass over the catabolic

activities of the fungi which give rise to a number of products of relatively small molecular weight, and in which the simpler plant acids predominate, and come at once to those groups which give evidence of their anabolic powers. These groups contain substances which, from their molecular weight and structure, present direct evidence of having been produced by synthetic processes. One such group of substances is presented by citric acid and its more complex derivatives. Citric acid is produced by a number of microfungi, in particular by *Aspergillus niger*, and also by a member of the wood-rotting fungi, namely, *Coniophora cerebella* (Fig. 1).

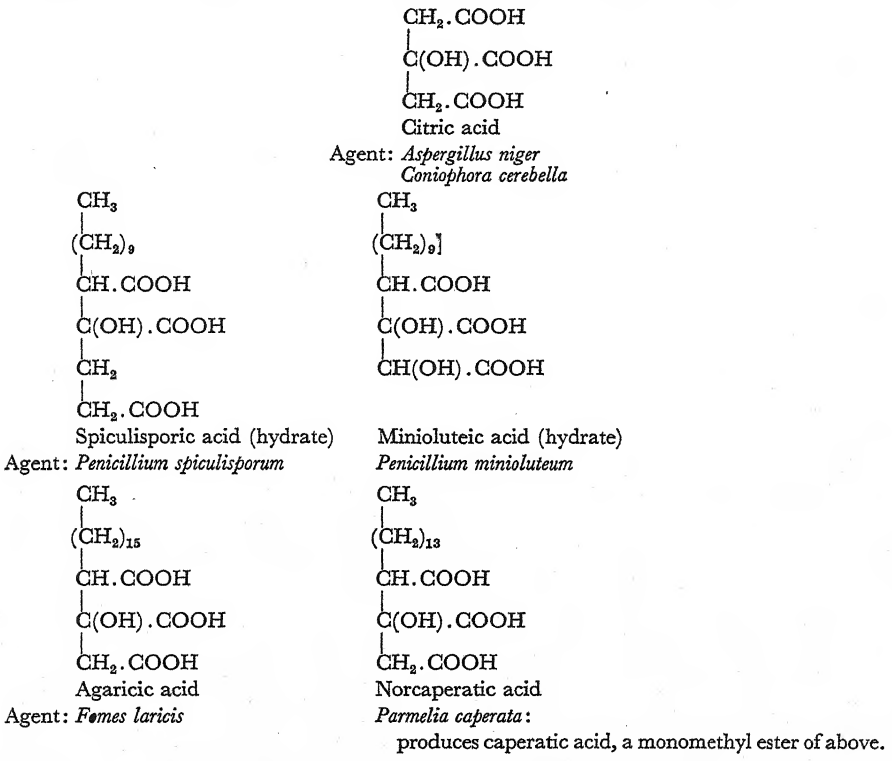
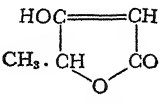


Fig. 1. Citric acid and related compounds produced by fungi.

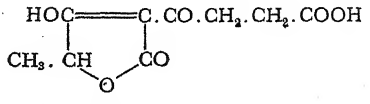
Spiculisporic and minioluteic acids are products of *Penicillium spiculisporum* and *P. minioluteum* respectively. Agaricic acid is a product of *Fomes laricis*. The crude fungus, or the crystalline acid extracted from it, is employed in pharmacy for the prevention of night sweats in phthisis. It has a strong cathartic action. Caperatic acid, a monomethyl ester of the norcaperatic acid shown, is a constituent of the lichen *Parmelia caperata*. The similarity in chemical structure between the members of this group is particularly interesting as showing the biochemical relationship between the higher and the microfungi and the lichens in which one of the symbionts is a fungus.

Another group of this type is seen in the acids of the tetronic acid series shown in Fig. 2.

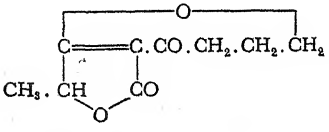
Five members of this series, namely γ-methyltetronic acid, carolinic acid, carolic acid, carlic acid, and carlosic acid, are metabolic products of *Penicillium charlesii*. Another member of the series, terrestric acid, an ethylcarolic acid, was obtained from *P. terrestre*. All these acids contain the same ring structure as ascorbic acid, which has recently been identified by Geiger and Galli as a product of *Aspergillus niger*. Penicillic acid, also showing in one of the forms allocated to it the tetronic acid ring structure, is possessed of marked antibacterial properties.



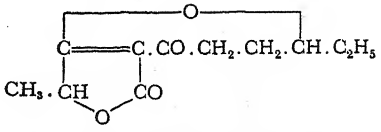
γ-Methyltetronic acid
Agent: *Penicillium charlesii*



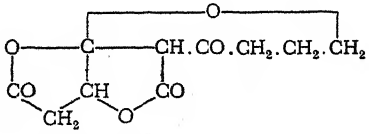
Carolinic acid
Penicillium charlesii



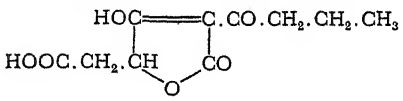
Carolic acid
Agent: *Penicillium charlesii*



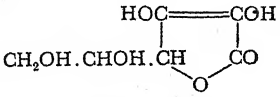
Terrestric acid (=Ethylcarolic acid)
Penicillium terrestre



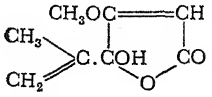
Carlic acid
Agent: *Penicillium charlesii*



Carlosic acid
Penicillium charlesii



Ascorbic acid (vitamin C)
Agent: *Aspergillus niger*

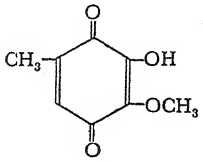


Penicillic acid
Penicillium cyclopium

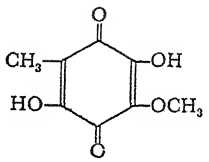
Fig. 2. Tetronic acid series of fungal products.

Another group of related synthetic products of fungal metabolism is found in the substituted benzoquinones (Fig. 3). Spinulosin and fumigatin were both obtained by Raistrick's group from their respective organisms, and shown to have antibacterial activity; fumigatin is particularly active in this respect. Phoenicin was first isolated by Friedheim; its constitution was determined by Posternak. The last of these products to be isolated, namely oosporein, was obtained by Kögl and van Wessem. It bears the same relation to spinulosin that phoenicin bears to fumigatin.

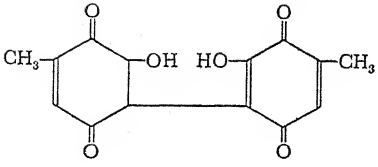
As a final example of these groups of related compounds the polyhydroxyanthraquinones may be mentioned (Fig. 4). They present a series



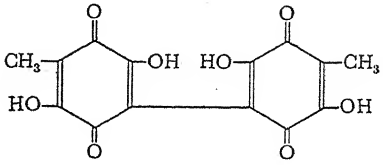
Fumigatin
Agent: *Aspergillus fumigatus*



Spinulosin
Penicillium spinulosum

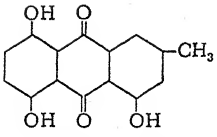


Phoenicin
Agent: *Penicillium phoeniceum*

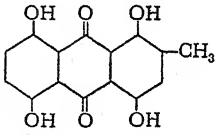


Oosporein
Oospora colorans

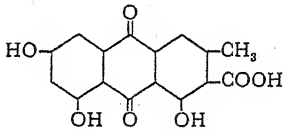
Fig. 3. Benzoquinone derivatives produced by fungi.



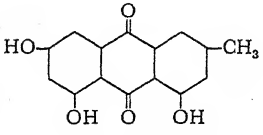
Helminthosporin
Agent: *Helminthosporium gramineum*



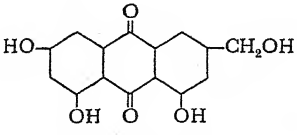
Cynodontin
Heminthosporium cynodontis



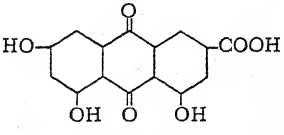
Endocrocin
Nephromopsis endocrocea (lichen)



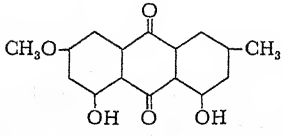
Frangula-emodin
Agent: *Dermocybe sanguinea*



Hydroxyemodin
Penicillium cyclopium
P. citreo-roseum



Emodic acid
Penicillium cyclopium



Physcion
Agent: *Aspergillus glaucus* series
Xanthoria parietina (lichen)

Fig. 4. Polyhydroxyanthraquinones produced by fungi.

showing interesting chemical relationships and link up the fungal products with those of the lichens and higher plants.

The next milestone in the investigation of the metabolic products of the fungi was the observation by Sir Alexander Fleming in 1929 of the production by a *Penicillium*, later identified as *P. notatum*, of a substance with antibacterial properties. The new product was termed penicillin. Clutterbuck, Lovell and Raistrick in 1932 showed that penicillin could be extracted from the culture solution by means of solvents but was of a somewhat unstable nature. Not until Sir Howard Florey and his co-workers at Oxford devised a laboratory method for the isolation of penicillin, admittedly in a very crude form, and obtained enough material for a few crucial clinical trials, were the immense therapeutic potentialities of penicillin glimpsed.

The further course of the development of penicillin is well known. The British chemical manufacturers who were straining every nerve to meet the demands of total war were unable to devote sufficient attention to the production of penicillin, but the American chemical industry, not so completely preoccupied, stepped into the breach, and by a magnificent technical achievement built up the production of penicillin into a successful industry. The methods worked out by the Americans became available to the British industrialists who, as their immediate war commitments fell away, were able to effect a rapid change over to penicillin production.

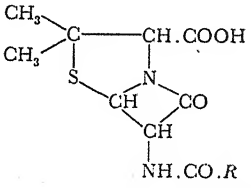
The development of the process of penicillin manufacture from the laboratory to the large-scale plant, bearing in mind the necessity of maintaining the purity of the cultures, and excluding adventitious organisms during the process and turning out a sterile product of high purity, packaged in carefully measured quantities of known potency, represents no mean achievement on the chemical engineering side. This achievement was, however, paralleled by important advances in the methods employed.

Perhaps the most radical advance was the change from surface culture to submerged culture. Although the submerged culture method for fungal fermentations had been employed from time to time in the laboratories and had even been investigated as a method for the manufacture of gluconic acid, this is the first time that it has been employed on a really large scale. In the citric acid fermentation, the details of which have been kept a closely guarded secret, it is generally accepted that the submerged culture method is not applicable, and that the process still retains the method of surface culture. It is obvious that a submerged method, if it can be successfully operated, results in a large saving of plant space and of labour, and offers less chance of contamination. Even those earlier plants, some of them in this country, where penicillin was originally made by surface culture are now giving way to the submerged process: in the matter of working costs the latter scores heavily.

Naturally the selection of the most active strain of the mould is a matter which has received careful attention, particularly in view of the fact that the best strain for surface work is not the best for submerged cultivation. Another factor of importance is the culture medium, many variants of

which have been tried. The addition of corn steep liquor, an improvement due to Moyer at Peoria, has proved of great advantage. The combined effect of all these improvements has been to increase the potency of the fermented liquor from 2–6 Oxford units per ml., as originally obtained at Oxford, to 350–400 units, which is now commonly attained on the 5000–10,000 gallon scale. In 100-gallon tanks over 1000 Oxford units per ml. have been achieved.

The chemical constitution of penicillin has at last been solved as a result of the joint efforts of organic chemists in many laboratories both in Britain and in the United States. A full account of this work has not yet been published, but from the summary available it appears that the general structure common to all the penicillins is as shown in Fig. 5.



(I) (F) *R* is Δ^2-pentenyl $CH_3.CH_2.CH=CH.CH_2-$
(II) (G) *R* is benzyl $C_6H_5.CH_2-$
(III) (X) *R* is *p*-hydroxybenzyl $HO.C_6H_4.CH_2-$
(IV) (K) *R* is *n*-heptyl $CH_3.[CH_2]_6-$

Penicillin
Agent: *Penicillium notatum*

Fig. 5.

The particular type of penicillin is determined by the nature of group *R*. The drug as marketed is a mixture of Na salts containing all the types, but the proportions vary with strain used and method of manufacture.

A unique feature of the molecule is the 4-membered lactam ring, not previously encountered in biological material. This ring is easily opened by chemical means or by the enzyme penicillinase and is the cause of the high instability of penicillin. Certain of the rearrangements which the molecule undergoes are very unusual and could scarcely have been predicted from the known reactions of organic chemistry.

Most of those organic chemists who have been concerned in the determination of the constitution of penicillin have also considered the possibilities of synthesis, but all attempts were for a long time fruitless. I understand that a minute amount of the pure crystalline sodium salt of penicillin II has now been obtained by synthesis. However, penicillin is now produced so cheaply by the biological method that its chemical synthesis has become more a matter of academic than of practical importance, at any rate for the present. There still remains the possibility of the chemical modification of biologically produced penicillin. Whereas penicillin I, II and IV do not appear to lend themselves to modification, the hydroxybenzyl side chain of penicillin III could probably be modified by the careful introduction of ether or ester groups without damaging the

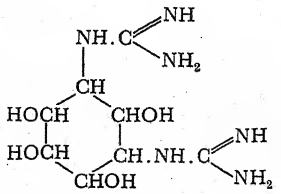
rest of the molecule. In this way it might be possible to obtain an active derivative partly synthetic in nature which would be less rapidly excreted than the natural product, which would be a distinct advantage in chemotherapy.

What was the cause of the long delay between Sir Alexander Fleming's original observation on the antibacterial effect of the contamination which invaded his Petri dish and the development and commercial production of penicillin? I think it was undoubtedly due to the fact that a third partner was now necessary in the research team. The mycologist and biochemist could not determine the chemotherapeutic applications of a fungal product without help from their medical colleagues. That is one reason why the development of penicillin may be termed 'epoch-making', using the epithet in its literal sense. Another reason is the fact that a huge new mould industry has been created to set alongside the fermentation citric acid industry—clear evidence to the man in the street of the practical importance of the fungi.

The spectacular success of penicillin has stimulated the search for other antibiotics which is proceeding in many laboratories throughout the world. A number have now been described and many have been chemically characterized, but in only relatively few cases has their constitution been determined. The most promising of the newer compounds so far obtained is streptomycin. In 1942 Waksman and Woodruff obtained from *Streptomyces lavendulae* an antibiotic streptothricin which promised well, but unfortunately exhibited a delayed toxicity to animals. A related organism, *S. griseus*, gave rise to a highly potent antibacterial substance, streptomycin, which was active against Gram-negative as well as Gram-positive organisms. It inhibited *Mycobacterium tuberculosis* at a dilution of 1 in 30,000.

When the earlier preparations were tested in vivo, unpleasant reactions were observed, but these have been minimized by the use of purer preparations. Streptomycin is possessed of very low toxicity. It has now been obtained in the crystalline form and shows great promise as an addition to the pharmacological armoury. It is too soon yet to assess its value in tuberculosis, but if the early successes claimed for it are substantiated it will indeed prove of great value in the control of this dread disease.

The chemical structure of streptomycin is now known in part. The general constitution of the molecule is that of a hydroxylated base (streptidine) attached through a glycosidic linkage to a disaccharide containing nitrogen (i.e. an amino-sugar). The streptidine moiety has the constitution shown in Fig. 6.



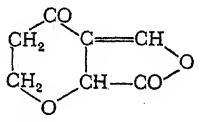
Streptidine
Agent: *Streptomyces griseus*

Fig. 6.

The constitution of streptidine is interesting as showing a relationship with meso-inositol. The amino-sugar portion of the molecule yields N-methyl-*l*-glucosamine on hydrolysis.

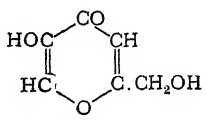
There is one other antibiotic substance to the structure of which I should like to call attention. That is the substance variously known as expansin, patulin, clavacin, clavatin or claviformin, according to the specific name of the organism from which it was isolated by its various discoverers.

Expansin, to give it its Dutch name, was first detected by Van Luijk as a product of *Penicillium expansum*, by its action in inhibiting the plant pathogen *Pythium debaryanum*. It is a powerful antibacterial agent inhibiting both Gram-positive and Gram-negative bacteria, and in an early test on naval personnel showed promising results in the treatment of the common cold. Later tests, however, gave negative results. The reason for this discrepancy is still not explained. The constitution of expansin, which was determined at the London School of Hygiene and Tropical Medicine, was found to be that of a substituted tetrahydro-γ-pyrone and in this respect it shows some resemblance to kojic acid which also has antibacterial activity (Fig. 7). During the war some of the Dutch clinicians gave further



Expansin

Agent: *Penicillium expansum* etc.



Kojic acid

Aspergillus flavus-oryzae group

Fig. 7.

attention to expansin and claimed for it modest successes in the treatment of mycotic diseases of the skin. It has also been suggested as a specific agent for combating the damping-off disease of seedlings caused by *Pythium*.

I have collected in Table 1 those antibiotic products of fungi so far isolated in crystalline or apparently pure form. Although most of them are antibacterial, some are antifungal in action. The number in the latter category is small at present, but is increasing more rapidly as the antifungal properties are attracting more interest. It will be noted that the table contains only products of microfungi. Investigation of the higher fungi is more difficult owing to their more exacting food requirements and slower rate of growth, but evidence is gradually coming to light that they do in some cases produce antibiotics as, for example, clitocybin, a product of *Clitocybe maxima* and *C. candida*. The comparison of the structural formulae of those antibiotics, of which the chemical constitution has been determined either completely or in part, does not lead to any very definite ideas as to the type of grouping in the molecule which produces the antibiotic action, because the types of chemical compounds differ considerably and appear to have little in common. Even the elements present show no regularity; in addition to carbon, hydrogen and oxygen, penicillin contains nitrogen and sulphur, streptomycin nitrogen only, expansin and many others no other elements. Geiger and Conn have sought to relate the antibacterial

Table 1. *Antibiotics derived from fungi.*

Name	Agent	Antibact. (AB) or Antifungal (AF)		Chemical information	Reference*
		AB	AF		
Actinomycin	*Actinomyces antibioticus*	AB	AF	Red plates, m.p. 250°, $C_{37}H_{50}O_{10}N_7$ (?). Possibly quinone type	Waksman & Tishler. *J. Biol. Chem.* CXLII (1942), 519
Aspergillic acid	*Aspergillus flavus*	AB	—	Cryst., m.p. 93°, $C_{12}H_{20}O_2N_2$. Constitution known	White & Hill. *J. Bact.* XLV (1943), 433
Citrinin	*Penicillium citrinum*	AB	—	Yellow cryst., m.p. 171°, $C_{13}H_{14}O_5$. Constitution known in part	Hetherington & Raistrick. *Phil. Trans. Roy. Soc.* CCXX B (1931), 269
Expansin	*P. expansum, P. patulum, Aspergillus clavatus*, etc.	AB	AF	Cryst., m.p. 110°, $C_7H_6O_4$. Constitution known	Birkinshaw, Bracken, Michael & Raistrick. *Lancet*, II (1943), 625
Flavacidin	*A. giganteus, A. flavus, A. parasiticus*	AB	—	Isolated as Na salt. A penicillin in which R = $—CH_2.CH_2.CH:CH.CH_3$	Fried, Koerber & Wintersteiner. *J. Biol. Chem.* CLXIII (1946), 341
Fumigatin	*A. fumigatus*	AB	—	Maroon needles, m.p. 116°, $C_8H_8O_4$, 3-hydroxy-4-methoxy toluquinone	Anslow & Raistrick. *Biochem. J.* XXXII (1938), 687
Geodin	*A. terreus*	AB	—	Pale yellow needles, m.p. 235°, $C_{17}H_{12}O_7Cl_2$. Constitution known in part	Raistrick & Smith. *Biochem. J.* XXX (1936), 1315
Gladiolic acid	*Penicillium gladioli*	AB	AF	Needles, m.p. 160°, $C_{11}H_{10}O_5$? Methoxy methyl-2-carboxyphenylglyoxal	Brian *et al.*, *Nature*, CLVII (1946), 697
Gliotoxin	*Gliocladium fimbriatum*	AB	AF	Cryst., m.p. 221°, $C_{13}H_{14}O_4N_2S_2$. Constitution known (?)	Weindling & Emerson. *Phytopath.* XXVI (1936), 1068
Glutinosin	*Metarrhizium glutinosum*	—	AF	Plates, m.p. over 300°. Possibly $C_{48}H_{60}O_{16}$.	Brian & McGowan. *Nature*, CLVII (1946), 334
Helvolic acid	*Aspergillus fumigatus*	AB	—	Cryst., m.p. 212°, $C_{32}H_{44}O_8$. Constitution unknown	Chain *et al. Brit. J. Exp. Path.* XXIV (1943), 108
Javanicin	*Fusarium javanicum*	AB	—	Red cryst., m.p. 208°, $C_{15}H_{14}O_6$. Probably a poly-hydroxy naphthaquinone	Arnstein, Cook & Lacey. *Nature*, CLVII (1946), 333
Kojic acid	*Aspergillus flavus-oryzae* group	AB	—	Cryst., m.p. 152°, $C_6H_6O_4$. 5-Hydroxy-2-hydroxy methyl-γ-pyrone	Yabuta. *J. Chem. Soc.* CXXV (1924), 575
Mycophenolic acid	*Penicillium brevicompactum*	AB	—	Cryst., m.p. 141°, $C_{17}H_{20}O_6$. Constitution known in part	Clutterbuck & Raistrick. *Biochem. J.* XXVII (1933), 654
Notatin	*P. notatum, P. resticulosum*	AB	—	Enzyme-glucose oxidase	Coulthard *et al. Biochem. J.* XXXIX (1945), 24
Penicillic acid	*P. puberulum, P. cyclopium*	AB	—	Cryst., m.p. 87° (anhyd.), $C_8H_{10}O_4$. Constitution known	Birkinshaw, Oxford & Raistrick. *Biochem. J.* XXX (1936), 394
Penicillin	*P. notatum, P. chrysogenum*	AB	—	Na salts of $C_9H_{11}O_4N_2S.R$. Constitution known	Fleming. *Brit. J. Exp. Path.* X (1929), 226
Puberulic acid	*P. puberulum, P. aurantio-virens*	AB	—	Cryst., m.p. 316–318°, $C_8H_6O_6$. Constitution known in part	Birkinshaw & Raistrick. *Biochem. J.* XXVI (1932), 441
'Puberulin'	*P. puberulum*	AB	—	Cryst., m.p. 220° (decomp.), $C_{17}H_{12}O_2N_2$. Constitution unknown	Campbell, Hirst, Foss & Jones. *Nature*, CLV (1945), 141
Puberulonic acid	*P. puberulum, P. aurantio-virens*	AB	—	Yellow prisms, m.p. 298°, $C_8H_4O_6$. Constitution known in part	Oxford, Raistrick & Smith. *Chemistry and Industry*, LI (1942), 485
Spinulosin	*P. spinulosum*	AB	—	Purple-black cryst., m.p. 203°, $C_8H_8O_5$. 3:6-Di-hydroxy-4-methoxytoluquinone	Birkinshaw & Raistrick. *Phil. Trans. Roy. Soc.* CCXX B (1931), 245
Streptomycin	*Actinomyces griseus*	AB	—	Basic. Forms cryst. salts. $C_{21}H_{37}O_{12}N_7$. Constitution mostly known	Schatz, Bugie & Waksman. *Proc. Soc. Exp. Med.* LV (1944), 66
Streptothricin	*Act. lavendulae*	AB	AF	Basic. Forms cryst. salts. $C_{13}H_{25}O_7N_5$. Constitution unknown	Waksman & Woodruff. *Proc. Soc. Exp. Biol. Med.* XLIX (1942), 207
Ustin	*Aspergillus ustus*	AB	—	Cryst., m.p. 184–6°, $C_{19}H_{15}O_5Cl_3$ (?) Constitution unknown	Doering *et al. J. Amer. Chem. Soc.* LXVIII (1946), 725
Viridin	*Trichoderma viride*	—	AF	Prisms, decomp. 217–223°, $C_{20}H_{16}O_3$ (?) Constitution unknown	Brian & McGowan. *Nature*, CLVI (1945), 144

* For further references see Oxford, *Annual Reviews of Biochemistry*, XIV (1945), 749.

activity of such products as expansin and penicillic acid to the presence of the $\alpha\beta$-unsaturated ketone grouping —CH=C—C=O. This group is capable of combining with sulphydryl compounds which are known to be of exceptional importance in enzyme systems either as constituents of the actual enzyme protein or in activators such as glutathione. The antibacterial action would thus be achieved by the interference with one of the essential enzyme reactions in the metabolic chain of the test organism. Although this may be the explanation of the activity shown in some cases it would not apply in all, since the $\alpha\beta$-unsaturated ketone group is not present in all fungal antibiotics, neither are they all inactivated by cysteine.

Now that we have briefly reviewed the antibiotics discovered to date, what of the future? Can we reasonably look forward to any advance on penicillin and streptomycin? To answer this let us look at the other side of the picture for a few moments and consider the disadvantages and drawbacks in the preparation and use of these substances.

Both penicillin and streptomycin require complex organic nutrients in the medium for high yield in addition to the sugars and inorganic salts which it contains. This is not of much consequence in the case of penicillin where the adjuvant corn steep liquor is a cheap waste product; but is much more serious from the point of view of cost in the production of streptomycin since the organism requires meat extract, an expensive form of food. Quite apart from the cost, however, the addition of such substances as these is a confession of ignorance. They obviously contain some essential foodstuff or foodstuffs, and the task of identifying it or them is one that must be solved before we can claim a full knowledge of the production process.

A potential danger in the use of antibiotics, and indeed of any type of antibacterial agent, is the possibility of the development of drug-resistant strains of the bacteria which we are fighting. This danger can be minimized by using large doses, but can be eliminated only by ringing the changes on the antibiotics employed. Further, allergic reactions are not unknown in penicillin therapy, the incidence of urticarial reactions being in some cases as high as 15%—another indication for changing the antibiotic. A minor disadvantage of penicillin as at present employed is the fact, already referred to, that it is a mixture of the sodium salts of at least four components, which of course have different antibiotic activities and antibacterial spectra. The proportions of the different components vary with the strain and method of culture of the organism. Thus it follows that in two batches of penicillin of equal potency as standardized against a particular organism (*Staphylococcus aureus*), the activities against other organisms may differ. Although the in vitro differences between the various penicillins are not striking, the chemotherapeutic efficacy, which depends not only on the activity in vitro, but on a number of other factors, does vary widely. Thus penicillin IV, probably owing to its greater rate of destruction in the body, is only about one-sixth to one-tenth as effective as penicillin II.*

This is a point of practical importance, for the strains of mould used by manufacturers up to 1944 yielded mainly penicillin II, but in the later

* From recent American work it appears that the therapeutic potency of penicillin IV is higher than the value indicated here, which is based on earlier observations.

commercial preparations a change has occurred in the direction of a fall in penicillin II and an increase in penicillin IV. It has been suggested that this change may be one factor in the decline since 1944 in the success achieved by penicillin against early syphilis. Now that attention has been drawn to the matter the manufacturers will no doubt be able to select conditions of fermentation which will favour the desired form of penicillin, and it may ultimately be feasible to separate the different forms of penicillin on a commercial scale so that we shall be able to select the most suitable chemotherapeutic agent for any particular purpose from a range of penicillins represented by pure chemical substances.

Sir Alexander Fleming himself has stated that it would be remarkable if the first useful antibiotic to be discovered should be the last or even the best. There is every encouragement to go forward in the quest for new antibiotics with the hope of providing at least a wider range of choice, and of discovering substances which in some particular respect are ahead of penicillin. The search should also reveal new substances of antifungal activity which may play a useful part in combating the human and animal mycoses, and others which will prove of value in agriculture, in suppressing fungus diseases of plants.

One of the newer methods of research, which has already yielded valuable results and which is capable of further development, depends on the induction of mutations in the fungi with resultant modification of their biochemical characteristics. In *Neurospora*, exposure to X-rays has resulted in the production of a number of distinct mutant strains in which the synthesis of various vitamins is deficient. In most cases the mutant character is inherited as if it were associated with the mutation of a single gene. Apparently the mutation of each gene causes a specific biochemical defect. The specific differences induced in this manner may take many forms, affecting both biochemical and morphological characteristics. One such form is an alteration in the quantitative relationships of the metabolic products. The effect of the irradiation on any particular characteristic of the fungus cannot of course be foreseen and may be advantageous or otherwise; it is only when the various mutants which arise at each exposure are tested biochemically that the results of this experiment can be appraised. The method has been applied to penicillin production for the attainment of high yields. The culture at present giving the highest yield of penicillin in the submerged process was derived in this way, being an X-ray mutant of *Penicillium chrysogenum*.

The special interest of the method lies in the potential value of its contribution to the study of fungal metabolism. By the use of such genetically altered forms it should be possible to isolate for examination one stage in the metabolic process, the principle being similar to the use of chemical inhibitors for certain enzymes in studying metabolism. Since the gene mutations are much more specific than chemical inhibitors the delicacy of the method should be considerably increased.

Another of the newer methods of biochemical investigation, which has already been applied to the study of animal and plant metabolic processes, is the use of tracer elements in the form of radio-active isotopes. The

principle is well known. It involves the replacement of the usual form of an element by one of its radio-active isotopes, which is of course chemically indistinguishable, but is tagged or labelled by its property of emitting radiations which can be detected by suitable apparatus. In this way it is possible to follow the fate of any particular atom in a molecule during its passage through the metabolic mill. Using a labelled carbon atom, direct evidence has been obtained of the fixation of carbon dioxide by the micro-fungi, an idea which was so revolutionary at the time it was first propounded in relation to the heterotrophic bacteria that it was accepted only with difficulty. The method should be capable of many further applications, and promises to throw considerable light on the mode of origin of the fungal products.

By the help of new techniques such as those outlined, and perhaps of others still to be evolved, our long journey towards the ultimate goal of a complete picture of the interrelationship of all the processes comprising fungal metabolism may be hastened. The possibility of the discovery of new products of value to man will provide an added incentive to progress. We are as yet only at the beginning of the journey. I would like, therefore, in conclusion to remind you of the well-known words of Sir Isaac Newton which may be deemed not inappropriate to the present situation: 'I seem to have been only like a boy playing on the sea shore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, while the great ocean of truth lay all undiscovered before me.'

MYCOLOGY AND PLANT PATHOLOGY

By W. C. MOORE
Plant Pathology Laboratory, Harpenden

In introducing the session on seed-borne fungi, Mr Moore said: I do not intend to stand between you and the speakers for more than a few minutes but, as was indicated in the preliminary announcement about these meetings, the papers selected were designed to show, as far as is possible in the time allotted, the relation of mycology to other subjects. This morning we are concerned with its relation to plant pathology, and I have been asked to make a few comments on this.

Now the relationship should not be difficult to define if everyone were agreed about what mycology and plant pathology are, or about the difference between a mycologist and a plant pathologist. But, unfortunately, in practice there seems to be a good deal of confusion about this, both nationally and internationally. In this country, where the study of plant diseases has, until comparatively recently, been dominated by investigations of plant pathogenic *fungi*, no clear distinction has been made between mycology and plant pathology, or between mycologists and plant pathologists. For very many years the official adviser in plant diseases to the Ministry of Agriculture and Fisheries has been called the Ministry's Mycologist, in contrast with the Entomologist, who was concerned with plant pests. Specialists in plant pathology at the Research Stations have generally been called Mycologists, and for twenty-five years there has been a special corps of Advisory Mycologists on the staffs of the Universities and Agricultural Colleges, who have been concerned with giving advice in the whole field of plant diseases. Change, if not clarification, is now taking place. At Headquarters there are posts for both Plant Pathologist and Mycologist, though the distinction is still a nominal one: under the new National Agricultural Advisory Service the corps of Advisory Mycologists has become an official corps of Advisory Plant Pathologists: and at the Research Stations and elsewhere there is a growing tendency to try to get away from using the terms 'Mycology' and 'Mycologist' with omnibus meanings. Abroad, the terms we have used are replaced by 'Plant Pathology' or 'Phytopathology' and 'Plant Pathologist', which cover more or less the same field, except that in certain countries plant diseases caused by eelworms fall within the scope of Plant Pathology, whereas in this country they are regarded as the concern of the entomologist and not of the 'mycologist'.

To me the relation between mycology and plant pathology presents no difficulty when a rational view is taken of the two subjects. Mycology is the science of *fungi* or, if you prefer, the branch of botany dealing with *fungi*, and those who study it are Mycologists. As such it comprises many different things, including the taxonomy of fungi, their collection and

preservation, their biology and nutrition, their uses in medicine and industry, and the part they play in diseases of plants, animals, insects and man.

Plant Pathology is a science treating of plant diseases, their nature, cause, progress and results: it is concerned with the pathological condition of plants, whatever the origin of that condition. The subject should therefore be regarded as comprising the study of plant diseases caused by fungi, algae, bacteria, viruses and eelworms; the study of plant pests; and of the vast field of nutritional or physiological disorders. These parts form a natural whole, and it is no longer possible to separate them, or to attempt to separate them, into watertight compartments. Entomology is bound up with the study of virus diseases; eelworms and fungi are often closely linked; and the incidence of both pest and disease is dependent on nutrition and environment. On the other hand, it does not follow that those who study plant pathology are necessarily to be labelled plant pathologists, in the way that I prefer to use the term. There is undoubtedly a place in advisory work for the general plant pathologist who is familiar with the nature and control of all the common troubles that lead to sickness in plants, but for the most part the subject provides common ground for the mycologist, entomologist, virus worker, helminthologist, bacteriologist, nutrition or soil chemist, and plant physiologist, each specializing along his own lines, but collaborating closely with cognate branches.

Thus we have two subjects, mycology and plant pathology, each with its many branches, and strictly speaking they overlap only where, and in so far as, each is concerned with plant pathogenic *fungi*. Hitherto the overlap has been an unduly large one in this country, for fungi as agents of disease have taken the lion's share of attention both in mycology and plant pathology. This predominance is likely to become less obvious in future. Already the increased attention given to virus diseases and nutritional disorders has shifted the emphasis somewhat in plant pathology, and the same will happen in mycology as soon as research in systematic, industrial, and medical mycology is given the attention it needs and deserves.

It was natural, in selecting a topic from the field of plant pathology for discussion to-day, to choose one from the overlap. Even so, the field was wide and the choice difficult. 'Seed-borne fungi' was given the preference, in part because it is of general interest and concerns most crop plants, and in part because of the opportunity to call on the experience of our neighbours in a matter of increasing importance, which has so far been given scant attention in this country. We are extremely glad to have with us to-day Dr Doyer, who so readily consented to give us the benefit of her long experience as Mycologist to the Official Seed Testing Station at Wageningen. As Chairman of the Seed-borne Diseases Committee of the International Seed Testing Association before the war, she repeatedly urged the need for testing seed for the presence of diseases, and for modifying the International Rules governing seed testing to achieve that object. Many of us, too, have found her beautifully illustrated *Manual for the Determination of Seed-borne Diseases*, issued in 1938 under the auspices of the International Seed Testing Association, most helpful and illuminating.

Prof. Muskett is Head of the Plant Diseases Division of the Ministry of Agriculture in Northern Ireland and occupies the chair of Plant Pathology at Queen's University, Belfast. He has devoted much of his time during the past twenty years to devising laboratory methods for seed examination for disease and to the control of seed-borne disease by chemical means. His contributions to the pathology of flax and cereal crops have indeed been notable ones. The third speaker, Dr Mary Noble, is on the Staff of the Scottish Seed Testing, Plant Registration and Plant Pathology Station near Edinburgh, where, as part of her duties, she has put her early training in seed-borne disease under Dr Doyer to good advantage. We had hoped to welcome Dr Paul Neergaard, Phytopathologist to the seed firm of J. E. Ohlsens Enke at Copenhagen, whose recent excellent Monograph on *Danish Species of Alternaria and Stemphylium* may be known to some of you, but he is at present in America and is not expected back until next May. And now I have much pleasure in calling on Dr Doyer to tell us of her experience in the determination of seed-borne disease.

SEVERAL SEED-BORNE FUNGUS DISEASES AND METHODS FOR IDENTIFYING THEM IN SEED TESTING

By LUCIE C. DOYER
Seed Control Station, Wageningen, Holland

It was a great honour and pleasure to me when I received your kind invitation to attend this Jubilee meeting of the British Mycological Society and to tell you something about the determination of seed-borne diseases.

In the year 1919 a special division for studying the sanitary condition of the seed was added to the Seed Testing Station at Wageningen. In the preceding years there had been severe outbreaks of *Fusarium* disease in cereals. It became evident, that such diseases gave much trouble in determining the germinating capacity of several kinds of seed, and therefore had to be studied thoroughly. The then director, F. F. Bruyning, took the initiative in starting a special division for this purpose. The task of this division was:

(*a*) to get acquainted with the symptoms of several seed-borne diseases by original research and by studying the international literature on the subject;

(*b*) to study various ways in which seed-borne diseases might be controlled or reduced; and

(*c*) to give information on these matters to those sending in seed samples.

In general, seed-borne diseases may be caused by:

I. Fungi.
II. Bacteria.
III. Animals (insects, eelworms, etc.).
IV. Viruses.

There is much to be said about these various kinds of infection and infestation, but on this occasion only the seed-borne fungus diseases will be considered.

Some of these fungus infections may be immediately recognized in the sample as it is sent, without using a lens or microscope. As such I mention sclerotia of ergot, of *Sclerotinia* etc., and bunt kernels in wheat, which are sometimes intermingled with the seeds, as well as distinct spots on the seeds being symptoms of fungus infection, e.g. spots caused by *Colletotrichum lindemuthianum* on beans.

For the detection of other infections, which are immediately recognizable in the sample, some magnification is necessary. This is so, for instance, with the pycnidia of *Septoria apii* Chester on celery seed, which can be recognized easily with the aid of a binocular microscope magnifying about twenty times. This fungus, causing Leaf Spot in the field, is very common on the seed.

As to the recognition of the infection with fungus spores (e.g. spores of *Tilletia caries* (DC.) Tul.), microscopical inspection is necessary. For this

purpose 100 seeds are shaken in a test-tube with water, which is afterwards poured into a porcelain dish. This liquid is partly evaporated upon a vapour bath until only a small amount is left, in which any spores present are in a more concentrated suspension. A drop of this suspension is then microscopically examined for the presence of bunt or other spores.

The majority of the fungus infections, however, are not immediately recognizable, but need the provision of special favourable conditions and some time to develop in their own characteristic way. Such favourable conditions are, as a rule, given to such infected seeds by a germination test, during which they are left in a moist atmosphere. For this purpose germination beds of white or grey blotting paper are used. To avoid mutual contact of the seeds as far as possible during such a health-germination test, galvanized zinc trays, with a perforated bottom on which a sheet of damp blotting paper has been spread and which are covered in the same way, have proved to be very practical. For several fungi a period of five to seven days is sufficient for these to develop in their own typical way, so that it becomes possible after that time to identify them.

Some fungi, as for example *Phoma betae* on beet seeds, need ten to twelve days to develop.

Those seed-borne fungi which develop from infected seeds in a moist atmosphere may be grouped into two types:

I. Those which penetrate deeply into the seeds and which cannot sufficiently be controlled by using common disinfectants.

II. Those which remain superficial and which, as a rule, can be controlled by seed treatment.

The fungi penetrating deeply are much less common than the more superficial ones. This is a very favourable circumstance, because in most cases it permits the discovery of fungus infection sufficiently early to make it possible to prevent damage in the field by treating the seed before sowing.

Among deeply penetrating fungi are those which cause the spot diseases on peas, i.e. *Ascochyta pisi* Lib. and two other closely related species, and on beans, i.e. *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav. and two species of *Ascochyta*.

Peas infected by *Ascochyta pisi* produce a white mycelium with brown pycnidia during the germination test. After about five days pink coloured tendrils, composed of thousands of bicellular spores, ooze out of the pycnidia. In our country and also, I believe, in yours, agricultural strains of peas are more resistant to this attack and do not as a rule show such a high percentage of infection as horticultural strains. For instance, in samples of Wonder of Witham, infections higher than 50% have been recorded. Among agricultural peas the little round smooth peas are more resistant than the larger wrinkled ones. Any lot showing too high a percentage of *Ascochyta* infection is rejected for sowing purposes by the 'General Netherland Inspection Service' (N.A.K.). During the past six years, however, other types of *Ascochyta* infection, which cause a foot-rot of plants in the field, have been found frequently on agricultural peas, though not in high percentages.

This foot-rot may be a result of infection by *Mycosphaerella pinodes* (Berk.

& Blox.) Vestergr. or by *Ascochyta pinodella* L. K. Jones, as originally described by Jones (1927). The mycelium growing out of such diseased seeds is not pure white but rather greyish; also the pycnidia are darker brown. Already in the germination stage the symptoms of the foot-rot manifest themselves, for the base of the rootlet and the stem of the seedlings becomes brown from the very beginning. As mentioned above, the cause of this foot-rot may be either *Mycosphaerella pinodes* or *Ascochyta pinodella*. Although the ascigerous stage of the first species is known, perithecia are never found on the germinating seeds, on which exclusively the pycnidial stage develops. The bicellular pycnidiospores are somewhat larger than those of *A. pisi*. Only the pycnidial stage of *A. pinodella* is known: these pycnidia contain one-celled spores of a smaller type than those of *A. pisi*. In our country *Mycosphaerella pinodes* is the prevailing species on the seeds.

Another deeply penetrating fungus infection is the spot disease of beans caused chiefly by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav. Whereas *Ascochyta* spots on peas are not easily detected on the dry seeds, *Colletotrichum* spots are often easily seen on dry beans as brownish grey spots with a lighter centre. They can be very conspicuous especially on white beans, and therefore diseased seed may be easily eliminated from the sample. By so doing it is possible to improve the quality of the seed sample. Beans with dark coloured seedcoats give much more difficulty, as the spots can hardly be recognized at once on them. The fungus develops quickly when the beans are put in a moist germination bed; after about five days, and with the aid of a binocular microscope, it becomes easily recognizable by the pink coloured spores, which by then have been formed abundantly, as well as by the dark setae, which encircle the acervuli.

Where the seed coat is dark coloured these acervuli are to be found best on the cotyledons under the seed coat. Less frequent spots on beans may be caused by two species of *Ascochyta*, i.e. *A. boltshauseri* Sacc. and *A. phaseolorum* Sacc. These fungi cause more reddish brown spots on the seeds, and in a moist atmosphere there are no setae. Pycnidia do not develop readily and as a rule it is not possible to say which of the two *Ascochyta* species has caused the spot. With these, as well as with those caused by *Colletotrichum lindemuthianum*, the same rule applies, namely, that the attacking fungi all penetrate deeply and cannot be controlled in a really efficient way by treating the seed.

Quite another type of deeply penetrating infection is represented by *Ustilago tritici* (Pers.) Rostr. as well as by *U. nuda* (Jens.) Rostr., causing Loose Smut in wheat and barley respectively. The mycelia of these smut fungi, having penetrated into the interior of the seeds, practically cannot be determined in a seed-testing station. Even if it were theoretically possible to examine a series of sections, the method would be far too slow. Therefore the diagnosis of this infection is exclusively a task for field inspection. In contrast to the other deeply penetrating infections mentioned, these smut infections can be controlled by means of hot water treatment.

The fungi which penetrate deeply are rare, and the majority of seed-borne fungus diseases are more superficial. These can be controlled efficiently by seed treatment.

First there is the *Macrosporium* infection on beans. The fungus in question, *Macrosporium commune* Rabenh. = *Pleospora herbarum* (Fr.) Rabenh., is usually located just beneath the micropyle, manifesting itself by a slight pink discoloration, for which reason it is called in the Netherlands 'red nose disease'. When such beans are put in a moist germination bed, this pink spot spreads quickly, and becomes purplish, surrounded by a yellow margin. Among the grey mycelium which develops in this region, dark multicellular spores are found sporadically. This fungus does not cause a disease with special symptoms in the field but very often it is the reason for young plants dying off in an early stage. If they happen to survive this dangerous stage, the seedlings grow into healthy plants. Therefore it is necessary to control this fungus by seed treatment. With beans, dusting is to be preferred to soaking. Treating the seeds with a solution may cause a swelling of the seeds, and afterwards a wrinkling of the seed-coat when they are dried again. In making comparative tests with dusted and not treated *Macrosporium* beans, the favourable result of treatment is often very striking. The dusted beans for the greater part retain their natural colour, and yield healthy seedlings, whereas the untreated seed show purplish discoloration and the developing rootlets are deformed.

The most frequent disease of cereals is the one caused by several species of the genus *Fusarium*. The prevalence of this *Fusarium* attack is very variable from year to year, depending on the weather during ripening and harvesting. If there is much rain in this period, infection can spread very quickly as has happened this year. When the fungus infects the kernel in the last stage of ripening it remains superficial: as soon as such kernels germinate, it causes a brown discoloration of the roots. On those roots only microconidia of *Fusarium* are to be found, so that it is not easy to determine which species is the cause of each special infection. The attack can be controlled efficiently by several disinfectants, the rootlets developing white and healthy after treatment. If, however, the kernels are infected by *Fusarium* in an early stage of their development, long before they are ripe, the fungus penetrates deeply into the seed. Such kernels as a rule lose their germination power completely. The percentage of such deeply infected kernels generally is much lower than the more superficially infected ones. In the germination beds the mycelium of such deeply infected kernels grows in a typical way, forming sporodochia with macroconidia, i.e. proper *Fusarium* spores, and it is then usually possible to determine which species is the cause. The most common ones are: *F. culmorum* (W.G.Sm.) Sacc. with violet mycelium and brown sporodochia composed of rather broad spores, *F. herbarum* (Cda.) Fr. with more slender spores, and *F. avenaceum* (Fr.) Sacc. with very slender spores formed in pinkish orange coloured sporodochia. The most pernicious species is *F. culmorum*, which causes the dying off of many young plants in the field. The kernels which have been infected in an early stage of their development cannot be cured by treatment. The power of germination, having been lost, is not regained after treatment: nor is the fungus killed, because the mycelium has penetrated too deeply into the seed.

Another way of testing the severity of *Fusarium* disease of cereals is to

sow 100 kernels in ground brickstone about 3 cm. beneath the surface. The seedlings have to pass through this thick layer in order to reach the open. Either the diseased seedlings are distorted and do not succeed in reaching the surface at all or, with less heavy infection, they grow into rather straight seedlings, which show a brown discoloured base. Though this method, the so-called Hiltner method, is a very reliable one, it is handicapped by the fact that it takes about two weeks to finish the test. Quicker results are required to satisfy those who send the seed.

In some years *Fusarium* disease may cause much damage in wheat, especially in summer wheat and rye. Oats and barley are not so often severely attacked. In connexion with this *Fusarium* disease, infection by *Gibberella zeae* (Schw.) Petch may be mentioned. Not only the conidial stage of this species with its typical *Fusarium* spores but also the ascigerous stage, characterized by blue perithecia, is easily formed on the kernels and on the blotting paper of the germination beds. In our country *Gibberella* infection occurs rather seldom, but on barley imported from America a rather severe infection has sometimes been found. This probably is due to the fact that *Gibberella* requires in general a warmer climate than ours. Sometimes *Helminthosporium* infection in barley may be found widely spread. On the kernels in the germination beds the long straight greyish spores can readily be identified with the aid of a binocular microscope. *H. gramineum* Rabenh., the cause of Stripe disease in barley, produces conidiophores and spores which are very similar to those of *H. teres* Sacc., the cause of Net Blotch. For this reason, it cannot be decided with certainty which of the two species is responsible for the infection. The spread of infection also seems to depend much upon the weather: in 1945, for example *Helminthosporium* infection could hardly be found at all on the seed, but this year it is widespread, so that the majority of the senders had to be advised, that only such samples as had received treatment could be used for seed purposes. The Wageningen Seed Testing Station advises that seed treatment is desirable when infection is only moderate, whereas it is considered essential when infection is strong.

Besides these two species of *Helminthosporium* there is a third, named *H. sativum* Pamm., King & Bakke which causes Spot Blotch of barley and which may occasionally be found on the seed. This species can easily be distinguished from the others with the aid of a binocular microscope, for the spores are glittering black and somewhat curved. In our country this form of infection is rare, but it may be found abundantly on imported barley. Its presence is thus to some extent an indication that the sample is not home-bred. On the other hand, *H. sativum* is found in our country more often on summer wheat, causing a foot-rot of the young plants.

A very common seed-borne infection, spread over a great part of Europe, is *Phoma betae* Frank on beet seed. It causes a root-rot in the field: when inspecting germinating beet seeds after some twelve to fourteen days, the majority of the rootlets are seen to be dark coloured. By using a binocular microscope many pycnidia are seen, with the spores oozing out abundantly in tendrils when placed in water. As a rule, the percentage infection is very high, often attaining 80 to 100%. When a germination test is made with

beet seed which has been treated, the majority of the rootlets remain healthy: the difference between treated and untreated seeds is very obvious. In the Netherlands treatment of beet seed is universal, and accordingly the recording of the presence of this fungal infection is no longer of any importance as one knows beforehand that a large percentage of *Phoma*-infected seedlings are usually to be found in the samples.

As to flax seed, there are two seed-borne fungi which may cause much damage in the field. The first is *Botrytis cinerea* Fr. f. *lini* van Beyma, which may be detected during germination tests by examining the germinating seeds after about five days. No lens is needed for this purpose. Much dying off in the field will result from this infection, which also spreads readily in the germination beds over the neighbouring healthy seedlings. The second one, *Colletotrichum linicola* Pethybr. & Laff., the cause of the flax Seedling Blight later on in the crop, can be detected only by means of a binocular microscope. In this way the acervuli on the infected seeds are easy to recognize. They contain a great quantity of pink, slightly curved spores, formed in groups with one or two setae in the centre. Both infections may be eliminated by seed dressing. Flax seed as a rule is not treated with a solution because of the difficulty of the seeds sticking together. Another fungus disease of flax, which is seed-borne is that caused by *Polyspora lini*, originally described by Pethybridge, Lafferty & Rhynehart (1921). At the Wageningen Station, however, we have not yet succeeded in identifying the symptoms of this infection either on seeds or seedlings.

Also on seeds of vegetables several diseases occur, which may become injurious to the crop.

Brassica seeds may be infected by *Phoma lingam* (Fr.) Desm., the cause of canker in the field. The infection is characterized by dark sunken stripes along the hypocotyl of the seedlings; but the symptoms do not appear if the seed has been treated in advance. This treatment is in common use in the cabbage-growing regions in our country. It is noteworthy that, since this treatment became so universal, the disease to-day is rather rare in the field as well as on the seeds.

Another infection, which may cause much damage some years, is the *Alternaria* disease of carrot. Recently *Alternaria* and related genera have been studied thoroughly by Neergaard (1945) in Copenhagen. He transferred *A. radicina* Meier, Drechsl. & Eddy, the species found on carrot seeds, to *Stemphylium radicinum* n.c., which conclusion I think is right. This species does not form chains of spores as does *Alternaria*, but the spores usually arise singly on the conidiophores, as in the genus *Stemphylium*. This fungus causes the seedlings to die in an early stage of development. During the germination test it also attacks the seedlings violently, causing mould growth. It can be eliminated completely by seed treatment. The harvested carrots also suffer from attacks by this fungus. Those which have been collected from a field derived from disinfected seed are much sounder and keep better than those originating from untreated seed.

In this paper I have given an account only of the most important seed-borne fungus diseases in our climate. There remain a number not mentioned. In international literature you will find lists recording a considerable

number of seed-borne parasites. To give a survey of this literature here is out of the question. I wish only to mention the name of W. L. Crosier, Mycologist in the Seed Testing Station Division of the New York Agricultural Experiment Station, Geneva, N.Y., who has done much research on this subject and who has studied several diseases on seeds, including those of plants growing in warmer climates. In future many seed-borne diseases, which are now still unknown, will certainly be recognized. This is a field for research in which there will be many interesting studies and on which international co-operation is much wanted. This is also the opinion of the International Seed Testing Association (I.S.T.A.), which opinion especially has been propagated during the international congresses of this Association, formerly held every third year in different countries. In the Committee on Determination of Plant Diseases of the I.S.T.A. there are eighteen members representing fourteen different countries. International rules for the determination of seed-borne diseases have not yet been given; they will, however, be discussed during the next congress of the I.S.T.A., which it is intended shall be held in the U.S.A.

As to the study of seed-borne diseases it was decided on a proposal made by the Vice-President of the I.S.T.A., Dr W. J. Franck, director of the Netherland Seed Testing Station, that an illustrated *Manual for the Determination of Seed-borne Diseases* should be compiled and published at the expense of the I.S.T.A. It was intended to be a guide for those seed-testing stations which wished to start with this work, but which had had little experience of it. This manual, provided with a series of drawings and photographs, was published in 1938.

International co-operation on the testing of the health condition of seed will probably also facilitate import and export of seeds. Fear of introducing infections and infestations from one country into another will be diminished, if there is a guarantee that measures will be taken to identify them beforehand and to eliminate them in some way. This is a suitable occasion for expressing this opinion and I sincerely hope, that many of you will give support to this cause by your co-operation.

REFERENCES

JONES, L. K. (1927). Studies of the nature and control of blight, leaf and pod spot, and footrot of peas caused by species of *Ascochyta*. *Bull. N.Y. (Geneva) St. agric. Exp. Sta.* no. 547, 46 pp.

NEERGAARD, P. (1945). *Danish Species of* Alternaria *and* Stemphylium. 560 pp., Copenhagen.

PETHYBRIDGE, G. H., LAFFERTY, H. A. & RHYNEHART, J. G. (1921). Investigations on flax diseases (Second Report). *J. Dep. Agric. Tech. Inst. Ire.* XXI, 169–75.

TECHNIQUE FOR THE EXAMINATION OF SEEDS FOR THE PRESENCE OF SEED-BORNE FUNGI

By A. E. MUSKETT
The Queen's University, Belfast

(With Plates I–III)

That seeds of agricultural and horticultural crops can carry fungi in the form of both mycelium and spores which may be parasitic and produce serious crop losses through causing disease is only too well known. To the category of the cereal smuts which may be taken as the classical example must now be added species of *Helminthosporium*, *Fusarium*, *Colletotrichum*, *Botrytis*, *Phoma*, *Septoria*, *Phialea* and many other fungi, while to the cereal hosts can be added the large majority of seeds of those species and varieties of plants used for cropping purposes. In many countries lists have been prepared of the seed-borne fungi with particular reference to those which can behave as parasites. In the Netherlands, Dr L. C. Doyer has made a special study of the problem as it is related to seed testing and has published her observations in her *Manual for the Determination of Seed-borne Diseases* (Doyer, 1938).

In Northern Ireland an attempt has been made, not so much to list those fungi which can and do behave as seed-borne parasites, but to devise rapid and accurate technique whereby seeds can be readily examined and their potential state of health determined in so far as it may be related to the presence of seed-borne parasitic fungi. It was decided that such technique should aim at providing results of both qualitative and quantitative value. The underlying reason for undertaking the work was as follows. Should it ever be considered that in the interests of crop hygiene some knowledge of the potential state of the health of seeds should be available in addition to data concerning their purity and germination capacity, then suitable technique to allow of this being done would be necessary. The methods employed would take their place as laboratory routine and would have to be capable of being operated by trained laboratory assistants. Experience has shown that the elaboration of such technique requires some research and patience, and that fresh difficulties are liable to arise whenever a new problem is approached. Nevertheless, it has also shown that these problems are eventually capable of solution and there appears to be no good reason why, in the course of time, satisfactory technique will not be built up which will allow of the speedy and effective routine examination of all seeds for their state of health whenever this may be necessary. Such an examination, taken together with relevant information concerning the growing crop from which the seed is produced (when this is required), should go far towards raising the standard of seed quality and thereby bringing about crop improvement. In all the methods so far devised the aim has been not only

to determine whether the parasite is present on the seed but also to ascertain whether or not it is present in a viable state.

For the purpose of this paper three particular examples have been selected and some account is given of the way in which the problems raised by each have been approached and dealt with in Northern Ireland. The examples are: *Helminthosporium* disease of oats; the seed-borne parasites of flax; and Blind Seed disease of ryegrass.

The Technique

Helminthosporium *disease of oats*

This disease is caused by *H. avenae* which is carried by the seed (usually on or in the glumes) as mycelium and sometimes as spores. The problem was to determine with accuracy all those seeds in a particular sample which carried the parasite. It has been shown (Muskett, 1937, 1938) that this may be done by sowing the seeds in soil under controlled conditions of temperature and moisture content and examining the seedlings for the presence of the disease, but this method was laborious and altogether unsuitable for the purpose required. Some other method of approach to the problem was necessary. An examination of the dry seeds is unsatisfactory, as spores of the fungus are seldom found and the resemblance of the mycelium of *Helminthosporium* to that of other dematioid species renders diagnosis by this means both dangerous and unreliable. It may be mentioned here that the colour of a seed sample does not form a reliable guide as to the extent of its contamination with *Helminthosporium*; some of the brightest samples of seed oats may be heavily contaminated, while in others which are dirty in appearance and of poor colour the fungus may be absent. Fortunately, it is possible to get the fungus to develop and sporulate on the seed, and it was decided to adopt the production of living spores by the fungus on the seed as the criterion for determining seed contamination. By plating out the seeds on damp filter-papers lining the bottoms of Petri dishes and incubating them for nine days at 22° C. the fungus was given the necessary conditions and time to sporulate, and it was found that, after cutting away the shoots, the seeds could be rapidly examined for the presence of spores using a wide field binocular microscope. Spore formation did not occur in every case of contamination and it sometimes did not take place freely, but by exposing the seeds for a short period to the light of a Hanovia quartz mercury vapour sun lamp in accordance with the suggestion of Dillon Weston (1933) it was found that spore formation was significantly increased. Even so, a few contaminated seeds may fail to show spore development, especially in freshly harvested samples. In seed samples stored over year in the laboratory the percentage of contaminated seeds has frequently been found to have increased after storage. Although this phenomenon has not yet been fully explained it would seem that after a period of rest under dry conditions, the mycelium of *Helminthosporium* attains a condition where it produces spores more readily when submitted to suitable conditions. In many seed-borne moulds the reverse is the case and a reduction in their incidence in a viable state is noticed with the

passage of time in storage. In spite of this slight disadvantage, whereby the estimate of the percentage contamination of a seed sample with *Helminthosporium* may tend to err on the low side, the method has given excellent results in practice and has been found to be quite reliable for the purpose of seed examination. It can be carried out by a trained laboratory assistant and it takes only nine days to complete the test, a period which is well within the limits required by seed testing for purity of sample and germination capacity.

But the recognition of seed contamination by *H. avenae* and the oat smut fungi may not complete the story of the seed-borne parasitic fungi affecting this crop. In Northern Ireland, experiments dealing with seed disinfection have always purposely referred to the effects of disinfection in general terms rather than ascribing its value solely to the control of *Helminthosporium* and smut diseases. This was because beneficial results were frequently obtained where neither *Helminthosporium* nor the smut fungi were present on the seed. In so far as the oat crop is concerned the role of the seed-borne species of *Fusarium* has yet to be clearly elucidated, and when this has been done it may be found desirable to examine seed samples for contamination with these fungi. The method employed for *Helminthosporium* may not be suitable for this purpose, in which case the elaboration of further technique will be necessary either to deal with *Fusarium* spp. alone or, better still, to serve for all the seed-borne fungi affecting this crop.

Details of the method

First day. The sample of seeds is thoroughly mixed and the number required for examination purposes selected by the method of random sampling. If a high measure of accuracy is desired the test will require 500 seeds, but this number may be scaled down according to the purpose for which the test is being made. Petri dishes (9·0 cm.) are prepared by lining the bottom of each with two filter-papers which are moistened after being placed in position, by almost filling the dish with tap water and allowing the surplus water to drain off after the papers have become saturated. Sterilization of the dishes is usually quite unnecessary. The seeds are arranged (fifteen to each dish) with equidistant spacing in the dishes and the closed dishes are then placed in an open container, the mouth of which is afterwards closed with cloths saturated with water in order to prevent drying out. The container is placed in an incubator set to run at 22° C.

Fourth day. The dishes are removed from the incubator, the lids are taken off and the seeds irradiated for 20 min. A Hanovia sun lamp operating at a distance of 1 ft. (30·5 cm.) from the seeds is suitable for this purpose (Pl. I, fig. 1). According to Dillon Weston (1936) spore formation is induced by intense white visible light and not by the invisible ultra-violet rays. The dishes are then repacked in the container which is replaced in the incubator.

Ninth day. The seeds are examined for the presence of conidia of *Helminthosporium avenae*. The shoots are first removed with a sharp scalpel in order to facilitate examination. A most suitable microscope to use for

this purpose is a wide field binocular model (paired eyepieces 15×, paired objectives 4×), giving a magnification of 60× (Pl. I, fig. 2). Only one examination of the seeds is necessary. The glume apices are first examined, then the basal end of the grain and finally the remainder of the grain surface (Pl. I, fig. 3).

Seed-borne parasites of flax

The principle of the Ulster method for the examination of flax seed for its contamination with seed-borne parasitic fungi (Muskett & Malone, 1941) depends upon the fact that the parasitic fungi in question can be relied upon to grow out from the seed into a nutrient medium. The problem was approached in several other ways, some of which could be successfully operated and some of which failed, but none of the methods investigated could compare with the one described in simplicity and ease of operation, both of which factors are of the greatest importance if technique of this type is to be translated into large-scale routine practice. By using this method the presence of the following parasites on or in the seed may be readily detected—*Colletotrichum linicola* (Seedling Blight), *Polyspora lini* (Stem Break and Browning), *Botrytis cinerea* (Grey Mould), *Phoma* sp. (Foot-Rot) and *Fusarium lini* (Wilt). No medium has been found to be more suitable for the work than 2% malt extract agar, which has proved to be satisfactory for all the parasitic types investigated. Furthermore, the test only required five days, a period which is well within the limits needed for practical seed testing. In seed samples heavily contaminated with moulds the identification of the organisms may prove a little more difficult than is normally the case, but this has not proved to be a serious obstacle, and when once a competent laboratory assistant has been trained for the work a high degree of skill and rapidity of working may be attained. More than 20,000 samples of flax seed have been examined in Northern Ireland by this method during the past six years and there has thus been ample opportunity for testing it on a large scale. Microscopic examination of the colonies is seldom necessary; a glance at the obverse and reverse sides of the dish and a rapid inspection by transmitted light is all that is normally required. When it is said that *Pullularia pullulans* and *Polyspora lini* may be quite readily distinguished by inspection of the cultures, some idea of the general reliability of the method may be gained.

Details of the method

First day. The seed bulk is thoroughly mixed and a random sample containing many times the number of seeds required for the test is placed in a large watch-glass. Petri dishes (9·0 cm.) are prepared as for normal culture work and a thin layer of 2% malt extract agar (1·5% agar agar) is poured into each. When the agar has set, ten flax seeds are spaced equidistantly on its surface, the usual precautions being taken to prevent contamination of the medium with extraneous organisms. If strict accuracy is required, 500 seeds are used for each sample, but this number may be reduced according to the interpretation to be placed on the quantitative value of the result. Each seed is picked up with blunt-nosed

forceps and dropped into position on the agar in the dish. The ends of the forceps are sterilized by dipping in methylated spirit and flaming after each ten seeds have been plated. This is because fungus spores are frequently present on flax seed, and it has been shown that there is a real danger of spreading contamination from seed to seed during the operation of plating unless frequent sterilization of the forceps is carried out (Pl. I, fig. 4). When plating has been completed the dishes are packed into an incubator set to run at 22° C. Great care and cleanliness must be continuously observed when this work is carried out constantly on a large scale over a prolonged period. Slovenly plating or the use of contaminated incubators may result in the necessity of repeating the examination of large batches of seed samples, particularly if contamination with such moulds as the *Monilia* stage of *Neurospora sitophila* or certain species of *Penicillium* become rife. For the decontamination and disinfection of incubators and small laboratories fumigation with formaldehyde, using a mixture of formalin (40%), and potassium permanganate has been found to be very effective.

Fifth day. The dishes are removed from the incubator and examined by eye inspection (Pl. II, fig. 5). Typical colonies of the five seed-borne parasitic fungi for which flax seed may be examined by this method are shown in Pl. III, figs. 9–13.

Blind Seed disease of ryegrass

The contamination and infection of perennial ryegrass seeds with *Phialea temulenta*, the organism responsible for Blind Seed disease, has assumed greater importance since the introduction of the more susceptible pedigree strains of this grass, and a simple method for the examination of seed samples for the presence of this parasite became necessary. Macrospores of the fungus are produced in abundance on the surface of the caryopsis of a contaminated seed and the observance of these spores forms a ready method for the examination of a seed sample. To make the examination each seed is placed in a drop of water on a clean glass microscope slide; one or both glumes are removed and the macrospores float out in large numbers into the surrounding water. Their presence is confirmed by a rapid microscopic examination of the slide using a magnification of 100×. Whereas this method has been found to be the most suitable and reliable for seed examination it cannot always be relied upon as an index of the viability of the fungus because the macrospores are comparatively short lived and usually lose their viability within six months of harvesting the crop. If, when the examination has been completed, the question of the viability of the fungus arises, information on this point can be gained by employing the method used by Neill and Hyde (1939) for the isolation of the fungus. The glumes are removed from the caryopses which are surface sterilized by immersion in 0·1% mercuric chloride for ten minutes. They are then washed in sterile water, bisected longitudinally and the portions plated out on 2% malt extract agar. The fungus is slow growing but if it is viable, recognizable colonies showing copious macrospore production will be observed after incubation at 22° C. for seven to twelve days according to the age of the seed. The culture method is not suitable for the estimation

of the percentage infection of ryegrass seed samples, because even when freshly harvested seed is used, where the fungus should be viable in every case, the estimate of infection will usually be found to be much lower than that given by microscopic examination. This may be explained in part by the killing of the fungus in some of the seeds by the process of surface sterilization and also by its non-exposure in some cases following upon the bisection of the seed. The microscopic method is therefore the most suitable for the routine examination of seed samples for percentage contamination, while evidence of the viability of the fungus may be obtained by the cultural method. Heavily infected seeds are normally opaque when examined by the diaphanoscope, and a suitably small sample for cultural work can be obtained by selecting these from the bulk with the aid of a diaphanoscope.

Details of the method

Microscopic method (for percentage contamination). The necessary number of microscope slides (3 × 1 in.) are cleaned and three drops of water are equidistantly spaced on each. Using forceps, one ryegrass seed from the thoroughly mixed sample is placed in each drop. Five hundred seeds are used when a quantitatively correct result is required. One or both glumes are removed from the seed while in the drop of water by using forceps and a dissecting needle (Pl. II, fig. 6). After each seed has been dealt with the ends of the forceps and the point of the needle are carefully cleaned by rubbing with a duster. When this operation has been completed the slides are microscopically examined for the presence of macrospores, using a magnification of 100 × (Pl. II, fig. 7).

Cultural method (for viability of the fungus). As this method is qualitative rather than quantitative the number of seeds used is not of such importance and will depend upon the circumstances of the test. Heavily contaminated seeds are very suitable and may be selected by the use of a diaphanoscope if available. The glumes are removed and the caryopses are surface sterilized by immersion for ten minutes in a 0·1% solution of mercuric chloride. They are then thoroughly washed in sterile water and bisected longitudinally, using a sharp scalpel which is sterilized at intervals as required and normally after dealing with each group of ten seeds. The portions are plated on 2% malt extract agar and are then incubated at 22° C. Colonies of *Phialea temulenta* will grow out from the infected seeds and can usually be recognized after seven to twelve days' incubation (Pl. II, fig. 8). As the fungus produces copious macrospores the observance of these serves as an aid to identification.

Technique for the Evaluation of Seed Disinfectants

The technique here described was not evolved in the first place with the express purpose of using it to examine seeds for the presence of seed-borne parasites; it was evolved for use in a series of tests designed for the rapid and accurate evaluation of seed disinfectants. These tests form the laboratory side of the work entailed in assessing the value of seed disinfectants

and they are operated on the following principle. It was assumed that if a method could be found for identifying the presence of a viable fungal parasite on a seed, and laboratory tests were made with this method using both non-disinfected and disinfected seeds, then a measure of the value of the disinfectant would be given by its effectiveness in suppressing the growth of the parasite being studied. This, indeed, proved to be the case and when the results obtained by the laboratory technique were compared with those obtained in the field over a three-year experimental period, a very high degree of correlation occurred between them. Thus it was found only necessary to experiment in the field with those disinfectants which show great promise as the result of laboratory testing and, even then, the reliability of the laboratory method allows the field work to be curtailed. This finding constitutes a very important economy both in time and expense, as the field side of such an investigation is a much more protracted and expensive undertaking. These results have been found to hold good in all the three examples under review, i.e. *Helminthosporium* disease of oats, the seed-borne parasites of flax and Blind Seed disease of ryegrass (Muskett, 1938; Muskett & Colhoun, 1942, 1943; Calvert & Muskett, 1945). By means of this laboratory technique a considered opinion upon the value of a seed disinfectant may be given within a period of a few days at any time of the year. Ineffective materials can be sorted out and discarded immediately and only those of the greatest promise need confirmatory testing in the field. The value of the application of this technique was clearly proved during the war when it was essential to try and find an effective seed disinfectant for flax. During the winter of 1939–40 it was found possible to investigate the behaviour of some hundreds of materials in the laboratory and as the result of this work a satisfactory disinfectant was found. Had field experimentation been the only method of approach to the problem, it would have taken a period of years to do the same work with only a proportion of the materials the values of which were assessed by this rapid technique.

Discussion

The work dealt with in this paper has been confined to the examination of true seeds for their contamination with seed-borne fungal parasites, but this forms only part of a much wider problem which is becoming of increasing significance in crop husbandry. Not only are seed-borne parasitic fungi responsible for crop losses; diseases caused by bacteria, deterioration by moulds, the carriage of viruses by the seed, damage by insects, deficiency diseases and physiological disturbances can all play their part by directly damaging the seed itself or by acting as the vehicle for the transmission of disease. There is also the grave danger of the transmission of parasitic organisms on detritus present as an impurity in a seed sample. Nor, with the rapidly increasing facilities for seed exchange, must the possible role of seed in spreading new diseases or making more widespread those which have become established, be overlooked. But crops are propagated by other means as well as by the use of true seeds, and these methods must also

be taken into account. Tubers, bulbs, runners, rhizomes, roots and other organs of vegetative propagation may play the same role as the true seed and the ensurance of their good health has, in many instances, already received greater recognition than with seed. Broadly speaking, one of the major problems to be faced in the future of crop husbandry may well be the taking of more effective measures to ensure the health as well as the viability of all those plant parts which are used for the purpose of crop propagation. In order that an adequate examination for health may be made, suitable technique for the purpose must be evolved, and when this has been done and a wide range of technique easy of operation and relatively inexpensive to practise has become available, the approach to the problem will have been very much simplified.

The need for seed surveys from the standpoint of seed health is a real one, and it may be that the carrying out of such surveys will prove to be a necessary prelude to the routine examination of seed supplies. The health survey made for flax seed produced in the United Kingdom may be quoted as an example. The recent emergency allowed of this being done, and for the past three years some 3000–4000 samples of flax seed from all parts of the United Kingdom have been examined annually by the Ulster method for their contamination with seed-borne parasites. The results have not yet been published, but they are of no little interest. Briefly, they indicate that contamination with *Colletotrichum linicola*, *Polyspora lini* and *Phoma* sp. is much heavier in the area to the north and west of the Pennines than to the south and east. Unless a succession of very favourable seasons is encountered, the build-up of parasites increases with repeated sowings of seed from the same stocks. This fact in itself explains why it is that the climate in the north and west is unsuitable for flax seed production in so far as it encourages a build-up of seed-borne parasites which may constitute a limiting factor in successful seed production. *When it is stated that the purity of all these seed samples was of a very high order and that the germination capacity in every case was greater than* 90%, *the significance of seed health as a factor which is not determined by routine methods of seed testing becomes very apparent. In accordance with present seeds legislation every one of these samples would be acceptable as seed of the first quality.* Had it not been for the fact that a method of seed disinfection was discovered in Northern Ireland which prevented completely any likely crop damage by Seedling Blight (*Colletotrichum linicola*) and largely prevented damage by Stem Break and Browning (*Polyspora lini*), the United Kingdom flax crop during the period of the war would have produced less fibre to the value of £2,000,000. The cost of making such a survey must be considered as trifling when it is realized that the expenses would be more than adequately covered by the saving of a few acres of crop in the light of the knowledge so gained.

The general distribution in the United Kingdom of seed oats contaminated with *Helminthosporium avenae* has yet to be determined, and the same remains to be done for ryegrass contaminated with *Phialea temulenta*. Already, useful information is accruing from the examination of ryegrass seed samples on a large scale for the presence or absence of this parasite. But, national boundaries will not serve the cause of science and this work

will not have been completed until it has been possible to make a thorough examination of seed stocks from crops grown over very wide territories—not, in fact, until such an examination has been made for every district and climate in which the crop is grown.

Finally, it is of interest to note that the technique here described was not deliberately sought with the object of seed examination for health and this in itself is a fact of no small significance. These methods were evolved during the process of endeavouring to discover rapid and accurate technique for the evaluation of seed disinfectants. Thus they assume a double importance because it has been shown in all the cases under review that, with but slight adaptation, they can be used to measure the value of a seed disinfectant as an instrument for the prevention of diseases likely to be caused by seed-borne parasites.

Summary

1. Suitable technique for the examination of seeds for the presence of seed-borne fungal parasites is described. Such technique must be easily operated on a large scale, relatively inexpensive, and capable of being exercised by trained laboratory assistants. The three particular examples for which the methods employed are described in detail are: *Helminthosporium avenae* of oats, the seed-borne parasites of flax and *Phialea temulenta* of ryegrass.

2. The following points are discussed: (*a*) The significance of examining seeds and other plant organs used in crop propagation, for their state of health. (*b*) The value of making seed health surveys by employing the technique described. (*c*) The limitations of routine seed-testing methods for the determining of purity and germination capacity in providing an index of the health of seeds. (*d*) The use of closely allied technique for measuring the values of seed disinfectants.

Due acknowledgement is made by the writer to his colleagues Dr John Colhoun, Mr Leonard Calvert, Mr J. P. Malone, Mr George Janeway and Mr Robert McIlwaine, all of whom have co-operated so wholeheartedly in this work.

REFERENCES

Calvert, E. L. & Muskett, A. E. (1945). Blind seed disease of ryegrass (*Phialea temulenta* (Prill. & Delacr.)). *Ann. appl. Biol.* xxxii, 329–43.

Dillon Weston, W. A. R. (1933). Sporulation of *Helminthosporium avenae* in artificial culture. *Nature*, cxxxi, 435.

Dillon Weston, W. A. R. (1936). The sporulation of *Helminthosporium avenae* and *Alternaria solani* in artificial culture. *Trans. Brit. mycol. Soc.* xx, 112.

Doyer, L. C. (1938). *Manual for the Determination of Seed-borne Diseases*. H. Veenman and Sons, Wageningen, Netherlands.

Muskett, A. E. (1937). A study of the epidemiology and control of *Helminthosporium* disease of oats. *Ann. Bot., Lond.*, N.S. i, 763–84.

Muskett, A. E. (1938). Biological technique for the evaluation of fungicides. I. The evaluation of seed disinfectants for the control of *Helminthosporium* disease of oats. *Ann. Bot. Lond.* N.S. ii, 699–715.

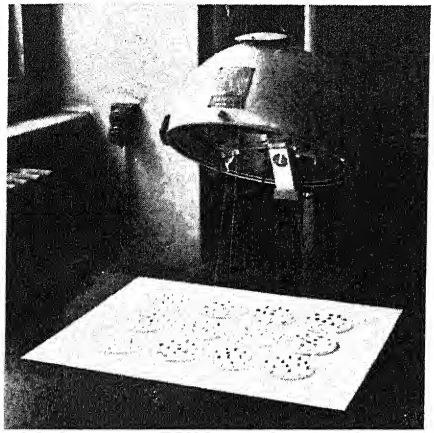


Fig. 1.

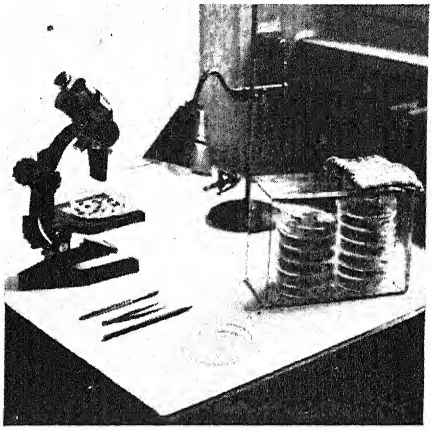


Fig. 2.

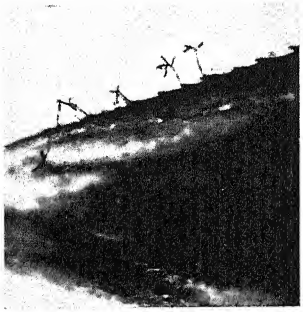


Fig. 3.

Fig. 4.

Fig. 5.

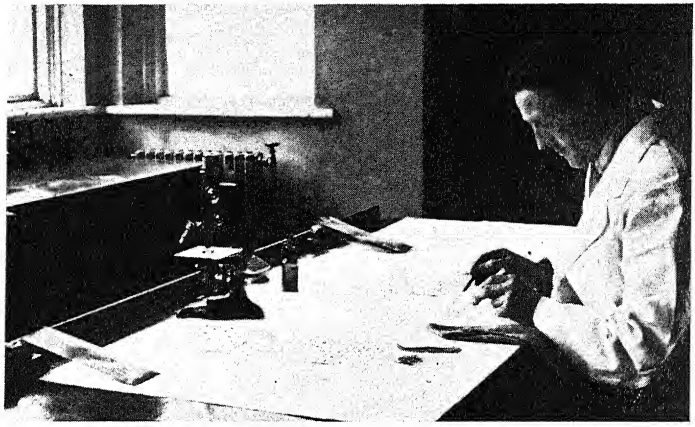
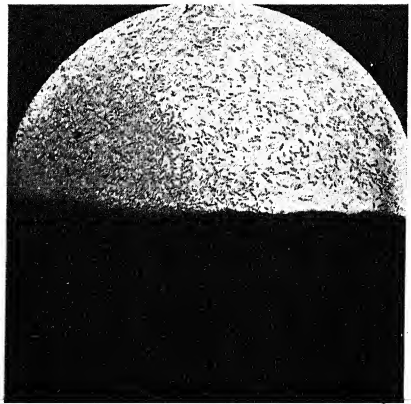
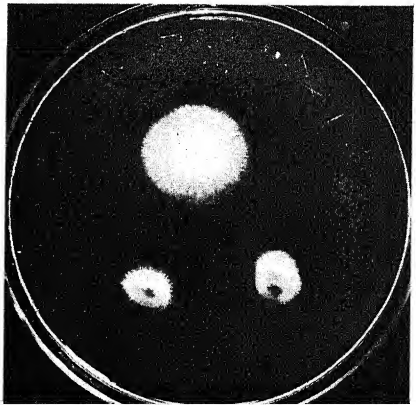


Fig. 6.





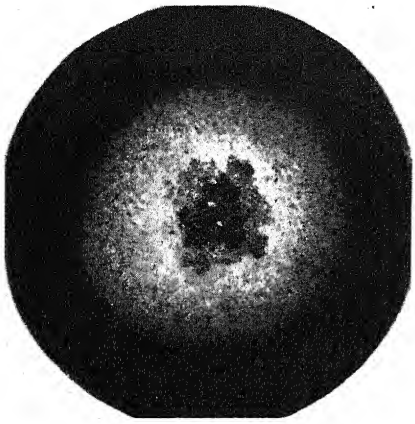


Fig. 9.

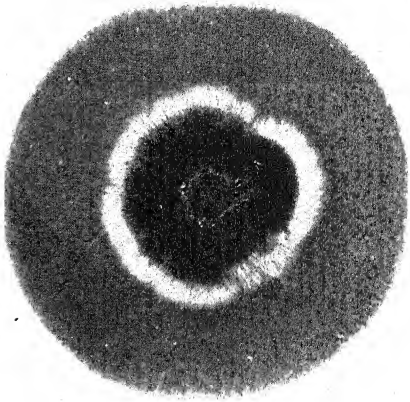


Fig. 10.

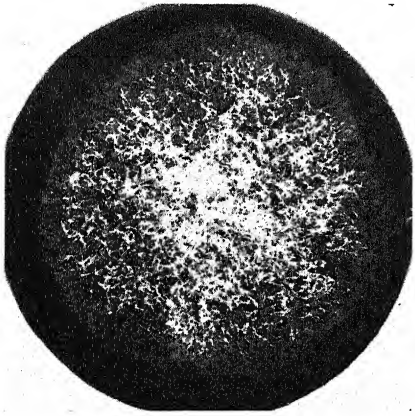


Fig. 11.

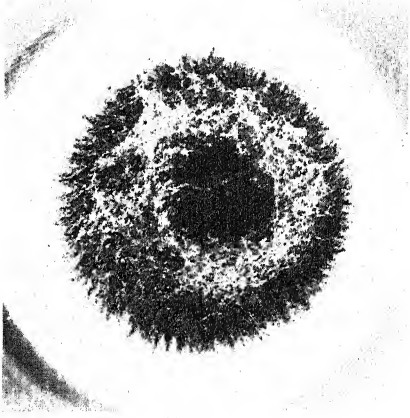


Fig. 12.

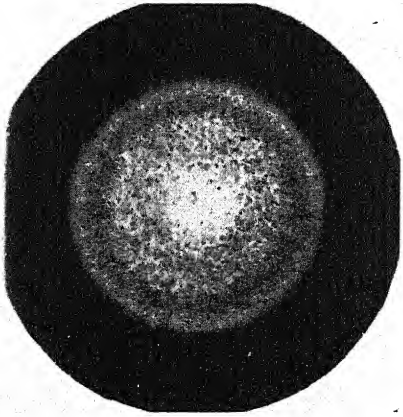


Fig. 13.

MUSKETT, A. E. & COLHOUN, J. (1942). Biological technique for the evaluation of fungicides. II. The evaluation of seed disinfectants for the control of seed-borne diseases of flax. *Ann. Bot., Lond.*, N.S. VI, 219–27.

MUSKETT, A. E. & COLHOUN, J. (1943). The prevention of seed-borne diseases of flax by seed disinfection. *Ann. appl. Biol.* XXX, 7–18.

MUSKETT, A. E. & MALONE, J. P. (1941). The Ulster method for the examination of flax seed for the presence of seed-borne parasites. *Ann. appl. Biol.* XXVIII, 8.

NEILL, J. C. & HYDE, E. O. C. (1939). Blind seed disease of rye-grass. *N.Z. J. Sci. Tech.* A, XX, 281.

EXPLANATION OF PLATES

PLATE I

Fig. 1. The method employed for irradiating seed oats to encourage the production of spores of *Helminthosporium avenae*.

Fig. 2. The apparatus and laboratory set-up required for the examination of seed oats for contamination with *H. avenae*. The museum jar shown in the foreground is used for storing the Petri dishes in the incubator.

Fig. 3. Photomicrograph of the glume apices of an oat seed upon which *H. avenae* is growing and sporing freely.

Fig. 4. The apparatus and laboratory set-up required for the plating out of flax seeds upon malt extract agar in carrying out the Ulster method for flax seed examination.

PLATE II

Fig. 5. The examination of the plates for the growth of seed-borne parasites of flax after an incubation period of five days.

Fig. 6. The apparatus and laboratory set-up required for the examination of ryegrass seed for contamination with *Phialea temulenta*.

Fig. 7. Macrospores of *P. temulenta* floating out from a contaminated seed into the surrounding water.

Fig. 8. Two typical cultures of *P. temulenta* (twelve days old) and a typical culture of *Pullularia pullulans* (five days old) are shown for purposes of comparison. *P. pullulans* is a common mould occurring on ryegrass seeds.

PLATE III

Figs. 9–13. Typical cultures of the five seed-borne parasites of flax which can be identified by the Ulster method. Fig. 9, *Colletotrichum linicola*; Fig. 10, *Polyspora lini*; Fig. 11, *Botrytis cinerea*; Fig. 12, *Phoma* sp.; Fig. 13, *Fusarium lini*.

SEED-BORNE DISEASES OF CLOVER

By MARY NOBLE
Plant Pathology Service, Department of Agriculture for Scotland

(With Plates IV and V)

I propose to limit the scope of this paper to the discussion of a small group of allied fungi, and the diseases of clover due to them. The fungi concerned include a species of *Sclerotinia*, hitherto described as a minor form of *S. trifoliorum*. I wish to compare this with *S. trifoliorum* Erikss., *S. minor* Jagger, *S. sativa* Drayton & Groves and *Botrytis anthophila* Bondarzew.

The fungus first referred to was described by Alcock (1928), when it occurred as a parasite of white clover seed and was tentatively identified as a minor form of *Sclerotinia trifoliorum* Erikss. I believe that it is a distinct species and propose to apply to it the specific epithet of *spermophila* for reasons which I shall give later.

S. spermophila was first found in white clover seed imported from central Europe and particularly from New Zealand; in these parcels many individual seeds were found which, to quote Mrs Alcock's excellent description, 'were characterized by their peculiar colour—a grey pink—which on examination under magnification × 16–20, proved to be due to the presence of the mycelium of a fungus occurring in grey shining flecks on the surface of the seed-coat' (Pl. IV, figs. 1, 2).

At that time, 1927, genuine Kent wild white clover seed commanded a very high price, and attempts were made to substitute seed of New Zealand origin which could be imported quite cheaply. The presence of these abnormal infected seeds was used as corroborative evidence in distinguishing between the spurious and the genuine English seed. Mrs Alcock records that they were then present to the extent of 4% by weight. Although they do not now occur in such quantities their presence is constantly noted in New Zealand seed by the analysts at the Scottish Seed Testing Station. It has extremely rarely been reported in English wild white seed and never in ordinary Dutch white or in red clover seed.

Infected seeds never germinate. If they are kept damp the resting mycelium develops and gives rise to a sclerotium (Pl. IV, fig. 3). In this process the rather flat-looking seed swells out, and after a week or so the testa at one point bulges and then breaks showing the black surface of the developing sclerotium, which then projects from the seed. On cutting through infected seeds which have been kept damp it is found that the fungus in giving rise to the black sclerotium has partially or wholly transformed the host tissue.

In my recent work on this fungus I have found that an imperfect stage, not recorded by other writers, sometimes appears on seeds thus kept damp. This stage bears a superficial resemblance to *Botrytis anthophila*. The

hyaline oval spores, measuring roughly 12 × 7μ, are formed in small groups of four or five on the swollen apices of the branched conidiophores (Pl. IV, fig. 5). The *Botrytis* stage of *Sclerotinia spermophila* is also formed on the germination of the ascospores (Pl. IV, fig. 4). If these are sown rather thickly on a plate of malt agar the conidiophores soon appear as little white pustules, and in these cultures a very sweet smell is present which has not been detected in any other culture. I am convinced that this imperfect stage was not overlooked by Mrs Alcock and her colleagues, or by Pape (1937), who had seed sent him by the late Dr Dorph-Petersen, but that, for one reason or another, it was not formed in their cultures. So far as I can discover no one else has worked with this fungus.

The fungus grows readily in culture, forming sclerotia similar to those on the seed. On malt-agar slopes growth often stops when half the medium is covered by sclerotia, *Botrytis* spores occur occasionally and microconidia are formed abundantly. The fungus seems to have a strong tendency to grow anaerobically, often forming sclerotia, conidia and microconidia under the surface of the medium. On oat-agar slopes growth continues until the medium is covered by sclerotia, both conidial forms being also present. Then after about six weeks apothecia are formed which mature in the test-tube (Pl. IV, figs. 6, 7). As Mrs Alcock has observed, the sclerotia do not seem to require a resting stage and this fungus has already proved useful to more than one teaching laboratory as all the stages of a *Sclerotinia* can be demonstrated in culture, the entire life cycle only occupying some six weeks or two months. The apothecia also appear readily if sclerotia are taken from culture, planted in sand and kept damp (Pl. V, fig. 8). *S. spermophila* is homothallic, as was proved recently by making single ascospore cultures and from them obtaining the perfect stage with normal viable ascospores.

The occurrence of an imperfect stage in this fungus is alone sufficient to differentiate it from *S. trifoliorum* Erikss., while the apothecia and sclerotia are markedly smaller.

S. trifoliorum is quite different in culture from *S. spermophila*. The former grows rapidly on malt agar at room temperature and soon produces a relatively small number of large sclerotia. At the same time characteristic dark mycelial bands appear stretching from the medium to the sides of the test-tube. Microconidia are not formed freely on malt agar but, as Keay (1939) has described, on oat agar they eventually appear abundantly as little cream-coloured masses. Apothecia apparently do not form in this fungus in culture. In his paper on Clover Rot, Pape (1937) says he believes that the fungus described by Alcock and Martin (1928) is not a variety of the Clover Rot fungus but a separate 'clover-seed *Sclerotinia*' ('*Kleesamen-Sclerotinia*'). He said he hoped so to describe it in a later paper, but I have been unable to discover whether he did so. I agree with Pape that this is not a minor form of *S. trifoliorum* but a distinct species. In morphology, however, it is similar to two other species of *Sclerotinia* occurring on the Leguminosae, *S. minor* and *S. sativa*. *S. minor* Jagger is usually associated with a disease of lettuce (Jagger, 1920), but has recently been recorded on red clover (Mujica, 1943), while *S. sativa* is described in America on alfalfa

and sweet clover (Drayton & Groves, 1943). Neither has been proved to be seed-borne. I am indebted to Dr J. Walton Groves for kindly sending me authentic cultures of these two species.

Apart from the fact that in *S. minor* no imperfect stage has been found, it resembles *S. spermophila*, although the asci and ascospores are smaller in *S. minor* which does not, moreover, form apothecia in culture. Mujica (1943) has recently recorded *S. minor* as causing a disease of red clover in Chile. In correspondence he wrote to me: 'Concerning *Sclerotinia minor* Jagger, it attacks in the same way as *S. trifoliorum* Erikss. I found it on red clover producing a neck rot which makes the plant look wilted as if it had been pulled off from the ground. Inside the decayed tissues, stalks and roots, there was a large number of minute sclerotia. Of course, this disease can be seed-borne, as most of these resting bodies are of the same size as clover seeds and it is not difficult to imagine that in the threshing process, stalks containing sclerotia can be disintegrated in the machine, the fungus being carried afterwards with the seed. As a matter of fact, I believe that this was the way in which this disease arrived in our country.'

In malt-agar cultures kept at room temperature *S. minor* grows rapidly, forming many small sclerotia very similar indeed to those of *S. spermophila*, but little white knots of hyphae among the sclerotia serve to distinguish the former at this stage. I have not found microconidia under such conditions and apparently apothecia of *minor* are developed only when the sclerotia are placed on damp sand.

S. sativa was described by Drayton and Groves (1943) on alfalfa and sweet clover and they state it 'closely resembles *S. minor* in culture. It can, however, be distinguished by the darker colour of the apothecia, the smaller asci and ascospores and by the fact that quite different conditions are required for the production of apothecia.' The apothecia in this species appeared only after the sclerotia had been exposed to low temperatures for a certain time and artificial spermatization had been carried out. This is in marked contrast to the ease with which they appear in *S. spermophila*.

There is one other fungus which in some respects resembles *S. spermophila* and that is the seed-borne fungus *Botrytis anthophila* described by Bondarzew (1914), Jaczewski (1916) and Silow (1933), and regarded as synonymous with *B. trifolii* of van Beyma Thoe Kingma (1927) and *B. antherarum trifolii* of Schlecht (1921). This is well known as an endophytic fungus in red clover. It apparently only behaves as a parasite in attacking the anthers, where in place of the pollen the grey *Botrytis* spores are formed. It does not seem to be common in England and has only been recorded on two or three occasions in Scotland, but it is evidently not uncommon in Sweden, occurring in stocks of the pedigree red clover seed produced there. The fungus has very beautiful fructifications, the spores on the anthers being rather long-oval and hyaline. Cultures can be made from almost any part of an infected plant or from the conidia, which can conveniently be obtained by keeping infected seeds in a damp atmosphere. On malt agar the fungus grows quite quickly, forming masses of greyish white conidia. The sub-aerial mycelium is very scanty and the fungus shows a strong tendency to grow anaerobically, even producing conidia and microconidia under the

surface of the agar. Bondarzew in describing this fungus writes that his cultures on bread developed a 'rosy tint'; Jaczewski on the contrary denies this: 'the rosy tint that Bondarzew mentions was never seen and was probably due to impurity in his cultures. I did not observe the pink colour on plates either.' Silow then comments that he also failed to see this rosy tint described by Bondarzew. Modern bread being rather unfavourable for work involving such colour values, I found a substitute in oat agar and in these cultures there is a very definite 'rosy tint' which has not appeared on malt agar, Dox agar, or potato dextrose agar. I venture to disagree, therefore, with Silow and Jaczewski and support Bondarzew. This rosy colour is, however, associated in oat agar with the formation of masses of microconidia. It is remarkable that microconidia were not apparently observed by the other workers. In my cultures on Dox agar they are formed fairly freely deep in the medium, but not evidently in sufficient numbers to cause the appearance of the pink colour. Silow describes and sketches the appearance of the fungus on this medium, but makes no remark about such microconidia which are of the typical *Sclerotinia* type, produced exogenously (Pl. V, fig. 10). Again, Silow comments that *Botrytis anthophila* grows so slowly in culture that after several months the colony scarcely exceeds 10 mm. in diameter, while I find that on potato dextrose the colony in less than two months covers a Petri dish 9 cm. in diameter, although on water agar at 16° C. *B. anthophila* makes very scanty growth, the conidia germinating to form microconidia almost immediately.

As already noted, the conidia of *B. anthophila* are formed readily in culture both above and below the surface of the medium. It is noticeable that while the conidia formed above the surface are similar in shape to those produced in the anthers, those formed under the surface are egg-shaped, much more like the *cinerea* type of spore (Pl. V, fig. 9). It is, I think, interesting to speculate whether the formation of conidia and microconidia in what are thus ostensibly anaerobic conditions may not be associated with the endophytic behaviour of the fungus in nature.

Silow states that he never found true sclerotia in his cultures. While that is generally true also of the series of cultures under discussion, in one or two made recently on malt agar a few sclerotia have appeared (Pl. V, fig. 11). The inoculum used was a mass of spores developed on a seed kept in a damp atmosphere. The spores of the *Botrytis* stage of *Sclerotinia spermophila* are not unlike those of *Botrytis anthophila* in shape and size.

It is difficult to avoid the conclusion that *B. anthophila* is the imperfect stage of a *Sclerotinia* and it may be significant that while *Botrytis anthophila* has never been found on white clover, *Sclerotinia spermophila* has never been found on red and is indeed unknown in the field, although it occurs so frequently in seed. The seeds infected by *Botrytis anthophila* are usually viable, in fact both Bondarzew and Jaczewski suggest that its presence may accelerate germination, while those infected by *Sclerotinia spermophila* fail to germinate. Some experiments at present in hand may throw light on the relationship of these fungi.

On account of the occurrence of the *Botrytis* stage *Sclerotinia spermophila* might have been included in the new genus *Botryotinia* as described by

Whetzel (1945), but in the sclerotia of *Botryotinia* the medullary hyphae are 'embedded in a hyaline flexible to gelatinous matrix' and there are no interhyphal spaces, while the conidial stage is of the *Botrytis cinerea* type. But the sclerotium of *Sclerotinia spermophila* appears to be of the *Sclerotinia* type, i.e. of 'densely interwoven hyphae with occasional small interhyphal spaces, hyphae not embedded in a gelatinous matrix', while the *Botrytis* stage is not of the *cinerea* type which Whetzel describes as having 'erect, fasciculate conidiophores usually more or less olivaceous often proliferating' etc. Again he remarks that 'homothallism seems to prevail in *Sclerotinia* while in *Botryotinia* heterothallism seems the rule'. *Sclerotinia spermophila* is homothallic.

According to Nannfeldt's classification the genus *Sclerotinia* falls within the family Ciborioideae in which the conidial forms are very variable (Nannfeldt, 1932). Other characters of this family are the positive reaction of the ascus pore to iodine and the structure of the excipulum and stem in which the inner hyphae are bounded by a layer turning at right angles at the stem. With all these points *Sclerotinia spermophila* agrees. Within the Ciborioideae Nannfeldt describes the genus *Sclerotinia* as 'with typical sclerotia, macroconidia, if present, of the *Botrytis* type'. On all points, therefore, this fungus agrees with Nannfeldt's definition of *Sclerotinia*.

It appears then that *S. spermophila* is a distinct species, and even if it should be shown later that it is identical with *Botrytis anthophila*, the presence of the perfect stage gives it precedence. The specific name has been chosen on account of the close association of these two fungi and because *Sclerotinia spermophila* is, as yet, not known to grow elsewhere than on seed.

In discussing *Botrytis anthophila* and *Sclerotinia spermophila* it has been pointed out that these fungi have, in the present investigation, exhibited features not described by previous workers. It may be significant, in this connexion, that the material of *Botrytis anthophila* used in the present work came from Sweden and was isolated from a pedigree stock of seed, while that used for culture work by the previous investigators was of Russian, Dutch and probably English origin. With regard to *Sclerotinia spermophila*, Mrs Alcock and her co-workers used New Zealand white clover seed, and Pape used seed from Scandinavia, but the cultures in which the *Botrytis* stage was recently found were made from seed purporting to be wild white clover grown in England.

These observations raise the question of variation in fungal parasites of such crop plants as white clover due to changing phenotypic or genotypic composition of the host plant. The case of *Sclerotinia bifrons*, recently described by Seaver (1945), indicates the confusion in the identification of fungal parasites and consequently in nomenclature due to such factors in the host plant.

The behaviour of these fungi of the *Sclerotinia* group as seed-borne organisms is interesting. *S. spermophila* has been so named because of its intimate association with seed: in fact, it is not known to attack or inhabit any other part of the plant and even the means by which it reaches the seed is, as yet, unknown. Like Blind Seed disease of ryegrass (*Phialea*

temulenta) this is therefore more accurately termed a parasite of the seed than a seed-borne parasite. It is unfortunate that this species was at first associated with *Sclerotinia trifoliorum* as it has led to the suggestion that the latter had been proved to be transmitted by means of mycelium within the seed. This possibility, I believe, has yet to be demonstrated, *S. trifoliorum* having only been proved to be transmitted by means of sclerotia which occur mixed with the seed. It should be emphasized at this point that neither Alcock and Martin (1928) nor Alcock (1928) stated that they found sclerotia of *S. spermophila* in and on the seed without keeping it for a time in a damp atmosphere, although Pape apparently did. It is possible, however, that the specimens he received from Dorph-Petersen were found during a germination test in the course of which the sclerotia had developed. The infected seeds cannot be separated from the healthy seeds by machining as they are so similar in size.

The occurrence of sclerotia of the Clover Rot fungus in seed samples has been repeatedly proved, and from observation of the seed passing through the Scottish Seed Testing Station, I would venture the opinion that its occurrence in this form is not uncommon. It is interesting to note that when Swederski (1924) recorded that sclerotia of *Sclerotinia* were thus found in clover seed in Poland, he was contradicted by Troussova (1926) on the grounds that it was impossible for them to get into the seed sample as they were formed exclusively on plants which were killed before the seed matured, and that these sclerotia were of *Typhula*. Pape (1937) and others have shown, however, that sclerotia can be formed some distance up the stems of infected plants and thus become mixed at harvest time with the seed from healthy plants. Although the majority of these sclerotia can be removed from seed samples by efficient cleaning, the smallest, which are about the size of wild white clover seed, remain behind. In the few experiments I have carried out with this fungus I have been impressed with its power of rapid growth in soil and I have been interested to note that Frandsen (1946) has recently stated that the fungus attacks primarily in this way, the mycelium developing directly from the sclerotium.

Seeds infected by *Botrytis anthophila* have so far proved indistinguishable to the naked eye, since there is no apparent colour change as with *Sclerotinia spermophila*. They are, however, easily detected if the sample is kept damp for a day or two when conidiophores but never sclerotia are freely formed. If seeds bearing fructifications of *Botrytis anthophila* are planted, infection is efficiently transmitted throughout the plant to the anthers. Infection may also be brought about artificially by sowing healthy seed on agar disks on which the fungus is growing, after the method used by Garrett (1936) working with *Ophiobolus*.

In conclusion, I would like to mention that *Botrytis cinerea*, recorded as attacking flowering heads of red clover, has been found by Mr Brett in the form of sclerotia mixed with seed. This fungus is apparently transmitted both in the form of mycelium in or on the seed and as sclerotia which look remarkably like those of *Sclerotinia trifoliorum*. Finally, I would like to take this opportunity of thanking my colleagues in the Seed Testing Stations of Cambridge and Edinburgh for providing me with much interesting material

used in this and other work on seed-borne diseases. I am also grateful to Prof. Sir William Wright Smith for generous assistance in preparing the Latin diagnosis.

Sclerotinia spermophila n.sp.

Sclerotia black, irregular in shape, often spherical, 0·5–1·5 mm. diam. Apothecia arising singly or in small groups from sclerotia, cup-shaped, becoming flat. Disk 0·5–1·5 mm. diam., cinnamon-brown (Ridgway). Stipe of the same colour as the disk at the top but darkening to black at the base, hairy, 1–9 mm. long, 0·5 mm. diam. Asci cylindrical 153–215 × 11–18 μ, average of fifty, 179 × 14 μ, apex slightly thickened and staining blue with iodine, spores occupying half the length. Ascospores 8, generally uniseriate, ellipsoidal, hyaline, continuous, 12–19 × 7–12 (13) μ, average of 100, 15 × 9 μ. Paraphyses branched at base, slightly thickened terminally, of the same length as the asci. Macroconidial stage of the *Botrytis* type, conidiophores usually arising singly bearing the conidia in small groups on short sterigmata. Conidia hyaline, oval to long oval, (7) 10–18 × (4) 6–10 μ, average of 100, 12·4 × 7·5 μ. Microconidia small, globose, approx. 3 μ diam., hyaline, with one guttule, arising exogenously on penicilloid conidiophores.

Hab. In seeds of white clover, particularly of New Zealand origin.

Sclerotinia spermophila sp.nov.

Species affinis *Sclerotiniae trifolii* Erikss. atque *S. minor* Jagger a quibus conidiis formam eorum in genere *Botryti* simulantibus differt.

Sclerotia nigra, ambitu irregularia, saepe sphaerica, 0·5–1·5 mm. diametro. Apothecia solitaria vel nunc e sclerotiis simul enata primum poculiformia deinde plana. Discus 0·5–1·5 mm. diametro, cinnamomeo-brunneus, stipite ad apicem similiter colorata sed basi nigrescente villosula 1–9 mm. longa, 0·5 mm. diametro. Asci cylindracei, 153–215 μ longi, 11–18 μ crassi (in medio 179 × 14 μ) apice parce incrassati, tincturam iodinam admittentes, ad medio sporis impleti. Ascosporae 8, plerumque uniseriatae, hyalinae ellipsoideae, 12–19 × 7–12 (13) μ (in medio 15 × 19 μ). Paraphyses basi ramosae, apice paulo incrassatae, asco aequilongae. Macroconidiorum origo ad modum generis *Botrytis*, conidiophoris plerumque solitariis, conidiis in soros parvos sterigmatibus brevibus dispositis, hyalinis, ovalibus vel ellipsoideo-ovalibus, (7) 10–18 × 6–10 μ. Microconidia pusilla, globosa, circ. 3 μ diametro, hyalina guttula unica praedita, conidiophoris penicilloideis suffulta, extrinsecus orta.

Hab. In seminibus *trifolii* repentis praesertim ex Nova Zelandia importatis.

REFERENCES

ALCOCK, N. L. (1928). Seed of *Trifolium repens* L. carrying a fungus resembling *Sclerotinia trifoliorum*, clover stem rot. *C.R. Assoc. Intern. Essais Semences*, VI, 31–36.

ALCOCK, N. L. & MARTIN, M. S. (1928). A seed-borne disease of clover. *Trans. Proc. Bot. Soc. Edinb.*, XXX, 332–7.

BONDARZEW, A. C. (1914). A new fungus disease of clover blossom. *J. Plant Disease* (Russian), VIII, 1–2.

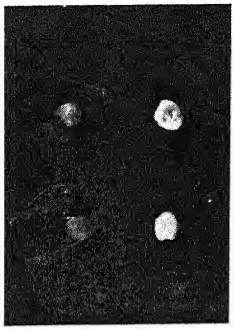


Fig 1. Fig. 2.

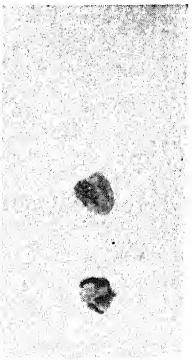


Fig. 3.

Fig. 4.

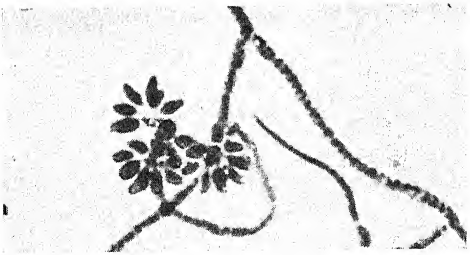


Fig. 5.

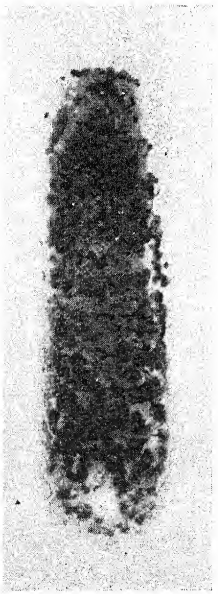


Fig. 7.

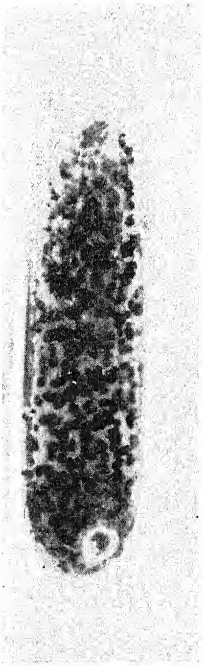


Fig. 6.

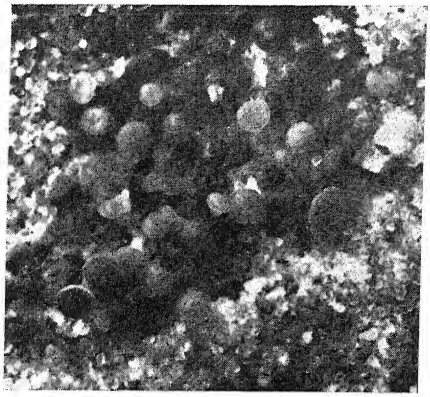


Fig. 8.

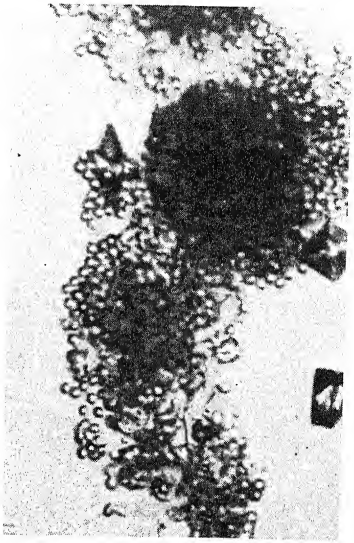


Fig. 10.

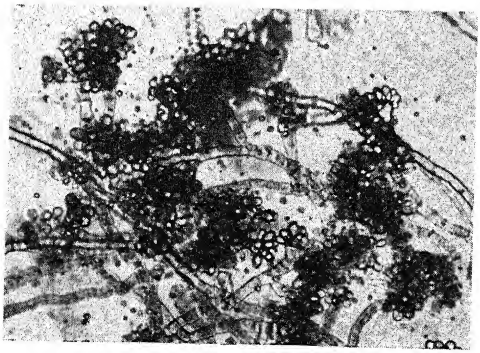


Fig. 9.

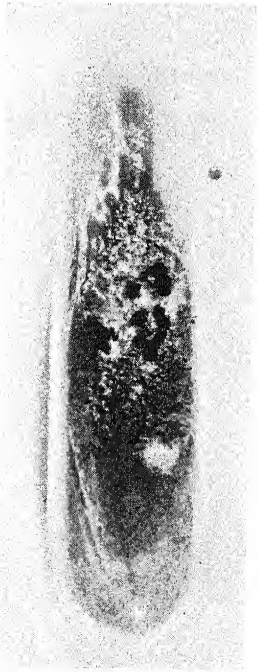


Fig. 11.

DRAYTON, F. L. & GROVES, J. W. (1943). A new *Sclerotinia* causing a destructive disease of bulbs and legumes. *Mycologia*, XXXV, 526.

FRANDSEN, K. J. (1946). *Studier over* Sclerotinia trifoliorum *Eriksson*. Kobenhavn (thesis).

GARRETT, S. D. (1936). Soil conditions and take-all of wheat. *Ann. appl. Biol.* XXIII, 667–99.

JACZEWSKI, A. A. (1916). Fungal and bacterial diseases of clover. *Mycol. Phytopath. Bur. Sci. Comm. Miny. Agric. Toula* (Russian), 31–37.

JAGGER, I. C. (1920). *Sclerotinia minor* n.sp. the cause of a decay in lettuce, celery, and other crops. *J. Agric. Res.* XX, 331–3.

KEAY, M. A. (1939). A study of certain species of the genus *Sclerotinia*. *Ann. appl. Biol.* XXVI, 227–46.

MUJICA, F. (1943). Chilean fungi not previously mentioned in the literature. *Bol. Sanid. veg. Chile*, III, 33–35. Abstract in *Rev. appl. Myc.* XXIII, 475.

NANNFELDT, J. A. (1932). Studien über die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. *Nova Acta Regiae Soc. Sci. Upsaliensis*, IV, Ser. 8, No. 2, 1–368.

PAPE, H. (1937). Beiträge zur Biologie und Bekämpfung des Kleekrebses (*Sclerotinia trifoliorum* Erikss.). *Arb. biol. Reichsanst. Berl.* XXII, 159–207.

SCHLECHT, F. (1921). Untersuchungen über die Befruchtungsverhältnisse bei Rotklee. *Z. Pflanzenzüchtung*, VIII, 152–7.

SEAVER, F. J. (1945). *Sclerotinia bifrons*. *Mycologia*, XXXVII, 641–7.

SILOW, R. A. (1933). A systemic disease of red clover caused by *Botrytis anthophila* Bond. *Trans. Brit. Myc. Soc.* XVIII, 239–48.

SWEDERSKI, W. (1924). Ueber Befall der Rotkleesamen mit Sklerotien der *Sclerotinia trifoliorum* Erikss. *Roczniki Nauk Rolniczych*, XI, 2.

TROUSSOVA, N. P. (1926). *Fungus Diseases of Red Clover* (Russian), Moskau. Land-Administr. Section, p. 96. Abstract in *Rev. appl. Myc.* VI, 98.

VAN BEYMA THOE KINGMA, F. H. (1927). Ueber eine *Botrytis*-Art von Rotkleesamen. *Meded. Phytopath. Lab. W.C. Scholten.* X, 37–41.

WHETZEL, H. H. (1945). A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate Discomycetes. *Mycologia*, XXXVII, 648–714.

EXPLANATION OF PLATES

Figs. 1–8. *Sclerotinia spermophila*

Fig. 1. Healthy seed of white clover (New Zealand).
Fig. 2. Seed of white clover (New Zealand) infected by *S. spermophila*.
Fig. 3. The same, with developing sclerotia, after being kept damp.
Fig. 4. Culture on malt agar developed from ascospores, showing *Botrytis* heads and sclerotia.
Fig. 5. *Botrytis* stage. (×340 approx.)
Fig. 6. Young apothecia in oat-agar culture.
Fig. 7. Mature apothecia in oat-agar culture.
Fig. 8. Apothecia in sand. (×2 approx.)

Figs. 9–11. *Botrytis anthophila*

Fig. 9. Conidia developed anaerobically in culture. (×170 approx.)
Fig. 10. Microconidia in Dox agar. (×500 approx.)
Fig. 11. Sclerotia in malt-agar culture.

RECENT ADVANCES IN THE STUDY OF TREE MYCORRHIZA

By ELIAS MELIN

Uppsala Universitets Institution för fysiologisk Botanik, Uppsala, Sweden

During recent years an increased interest has been shown in mycorrhiza, especially that of forest trees. It has therefore seemed to me suitable on this occasion to summarize the results obtained in the study of these problems during the last few years. For natural reasons I shall in the first place touch upon the investigations carried out in my laboratory in Uppsala.

I take it for granted that the appearance of the ectotrophic and ectendotrophic types of tree mycorrhiza is well known to this audience. As a result of infection by certain fungus mycelia occurring in the soil the short roots thicken and ramify in a characteristic manner, often very vigorously. Furcation commonly occurs in the pine, while the ramification in other trees, e.g. spruce, beech, birch, oak, is generally monopodial. In colour, mycorrhiza is often yellowish to darkish brown. Other colours may, however, also occur, such as yellow, white, rose, bluish, violet or black. Forms of mycorrhiza that possess different colours are frequently found alongside one another. Our experimental work has enabled us to establish the fact that the colour of mycorrhiza depends in the first place on the fungal symbionts and that different mycorrhizal fungi can produce different colours.

The anatomical structure of tree mycorrhiza is characterized, above all, by the powerful development of hyphae between the cortical cells and by the mycelial mantle round the roots. Often, most of the short roots of the trees are found to be converted into mycorrhiza. It is, therefore, not astonishing that this condition has long been a subject of scientific interest.

In the early literature, contradictory opinions are found as to the nature of tree mycorrhiza. Some investigators maintained the view, first pronounced by Frank in the eighties of the last century, that the two components, roots and hyphae, live in mutualistic symbiosis, i.e. that they mutually favour each other. Others considered that the mycorrhizal fungi are of no importance for the higher symbiont's absorption of nourishment but, on the contrary, are injurious parasites. During the last few decades, however, facts have accumulated which conclusively show that the mycorrhizal fungi are beneficial or even necessary for the trees. In 1917 I demonstrated that in certain recently drained bogs in North Sweden seedlings of pine and spruce could develop only when they had mycorrhiza (Melin, 1917). I found seedlings with and without mycorrhiza side by side with one another. The former were green and luxuriant, the latter yellowish green and poorly developed. Similar observations were subsequently made in various treeless regions in the world. It was found that coniferous plants thrive in such regions and develop normally only

when mycorrhizal fungi are present in the soil and thus enable mycorrhiza to be formed. This applies, for instance, to certain sections in Southern Rhodesia, Australia (Kessel, 1927; Clements, 1938), the Philippines (Oliveros, 1932) and North America (Hatch, 1936; White, 1941).

The question of the importance of tree mycorrhiza cannot be studied with any great success until we have learnt to know what the fungal symbionts are. After these have been obtained in pure culture we can conduct cultural experiments with tree-seedlings under different conditions with and without mycorrhizal fungi. It will also be possible to study the physiology of the mycorrhizal fungi.

The first biological analyses of tree mycorrhiza were carried out twenty-five years ago (Melin, 1921). From mycorrhiza of pine and spruce I then isolated a number of fungi and, with these, mycorrhizal syntheses were afterwards carried out in pure culture. The synthetic mycorrhiza had the same appearance and structure as that from which the fungi had been isolated.

From these experiments it was already seen that mycorrhiza is formed with pine and spruce by several different species of fungi. This has since been confirmed. It was further established that most of the isolated fungal symbionts belonged to the Basidiomycetes (Melin, 1923*a*). They formed no sporophores in pure culture, however, and could therefore be identified only in a few cases. By means of Buller's (1931) method it was possible to identify some mycelia that had been isolated from nodule mycorrhiza of pine in various localities in the Uppsala district. These proved to belong to different *Boletus* species, namely *B. luteus*, *B. variegatus* and *B. granulatus*. Further, the yellow mycorrhiza-forming mycelium that had been isolated from the common yellow-coloured pine mycorrhiza could be determined with a high degree of probability as *Corticium sulphureum* Fr. (= *C. croceum* (Kze) Bres.).

After isolation experiments had shown that the mycorrhizal fungi of the trees were more especially to be found among the Basidiomycetes it was a ready assumption that many of the Hymenomycetes growing in forests are mycorrhiza producers. In order to ascertain how matters really stood in this respect I and my pupils (chiefly A. B. Hatch and O. Modess) isolated in pure culture a large number of common forest fungi for the purpose of conducting experiments with them in synthetic formation of mycorrhiza. From these experiments it became evident that mycorrhiza-forming fungi are to be found among the Hymenomycetes as well as the Gasteromycetes. Among the Hymenomycetes mycorrhizal fungi have hitherto been experimentally demonstrated within the following genera (Melin, 1922, 1923*b*, 1925, 1936; Hatch & Hatch, 1933; Doak, 1934; Modess, 1941): *Amanita*, *Boletinus*, *Boletus*, *Cantharellus*, *Clitopilus*, *Cortinarius*, *Entoloma*, *Lactarius*, *Russula* and *Tricholoma*. In addition to these there are probably several other genera, for instance, *Gomphidius*, *Hebeloma*, *Hydnum*, *Hygrophorus*, *Inocybe* and *Paxillus* which contain mycorrhizal fungi. Examined species belonging here, however, have proved to be very difficult to isolate in pure culture, and it has therefore not been possible to make unexceptionable synthetic experiments with them. Among the Gastero-

mycetes only *Rhizopogon* and *Scleroderma* have so far been demonstrated to contain mycorrhiza-forming species (Doak, 1934; Modess, 1941).

Hitherto about fifty Basidiomycetes (of these five *Amanita*, sixteen *Boletus*, three *Cortinarius*, five *Lactarius*, seven *Tricholoma* species) have been experimentally proved to be mycorrhiza-formers on forest trees. Undoubtedly this number will be increased as a result of future investigations. Probably it will be found that several genera of the Hymenomycetes, such as *Amanita*, *Boletus*, *Cortinarius*, *Lactarius* and *Russula*, exclusively or predominantly, contain mycorrhizal fungi. Others again, such as *Tricholoma*, seem to contain, besides mycorrhizal fungi, numerous saprophytic species (e.g. *T. brevipes*, *T. nudum*, *T. fumosum*, *T. personatum*), which are to be regarded as litter-decomposing species. Still others, finally, such as *Clitocybe*, *Collybia*, *Marasmius* and *Mycena*, perhaps contain exclusively saprophytic species. The hitherto examined representatives of these genera decompose the celluloses and lignins of the litter and in that way play a great part in soil ecology (Lindeberg, 1944).

Whether the Ascomycetes are also able to form mycorrhiza with trees has not yet been established with certainty. This is, however, probably the case. Lihnell (1942) has shown that *Cenococcum graniforme* forms mycorrhiza with conifers as well as deciduous trees. This fungus occurs very abundantly in various forest soils as sclerotia of variable size (Ferdinandsen & Winge, 1925). Its sporophore is as yet unknown, and hence its taxonomic position cannot be determined. Probably, however, it belongs to the Ascomycetes (Lihnell, 1942). It develops a jet-black mycelium (= *M.R. nigrostrigosum* Hatch), and the mycorrhiza formed by this is also black with abundant radiant hyphae (Hatch, 1934). This type of mycorrhiza is especially common in nature.

Some mycorrhiza-forming species of fungi are highly specialized, forming as they do mycorrhiza only within a certain genus of trees. Others are less specialized and can produce mycorrhiza in different genera (Melin, 1925). To the most highly specialized belongs *Boletus elegans* which occurs only on larch. To the somewhat less specialized belongs, for instance, *Lactarius deliciosus*, which forms mycorrhiza with pine and spruce. To the least specialized belong, amongst others, *Amanita muscaria* (shown to be symbiotic with pine, spruce, larch, birch and others) and the just-mentioned *Cenococcum graniforme* (demonstrated as symbiotic with pine, spruce, birch, aspen, lime-trees, etc.). This specialization of certain mycorrhizal fungi has not yet been satisfactorily elucidated. Possibly it depends on substances which are excreted from the roots and which exercise an antibiotic effect on certain fungi.

It is remarkable that so many species of fungi, some of which are but little related to one another, form mycorrhiza with one and the same species of trees. In *Pinus silvestris*, which has hitherto been studied most thoroughly in this respect, about thirty species have so far been proved to be mycorrhiza-formers. The actual number is no doubt many times higher. It is probable that the fungal symbionts of the ectotrophic and ectendotrophic mycorrhiza possess certain similarities in respect to physiology. A close study of the physiology of the mycorrhizal fungi is therefore of great interest.

According to recent investigations, among the mycorrhizal fungi of trees there are species with different requirements of soil activity. This aspect of the subject was investigated by O. Modess (1941) on a number of known mycorrhizal fungi in Swedish pine forests. These preferred a soil with an acid reaction, even though different species showed different growth optima. To the very acidophilic belong certain *Amanita* species (*A. muscaria* and *A. porphyria*), while *Boletus granulatus* prefers a slightly acid reaction. Among mycorrhizal fungi of trees there are probably also some species that prefer a neutral or slightly alkaline reaction. It has been reported that a pH value approaching neutrality does not inhibit the development of mycorrhiza on pine in Australia (Cromer, 1935).

The mycorrhizal fungi of trees are favoured by certain vitamins if they are grown on a synthetic substratum in pure culture (Melin and associates, 1939, 1940, 1942). In many cases they have entirely lost their own capacity of synthesizing one or more of these vital substances. It is of interest to note, however, that different species differ very much in this respect. A simple type is represented by *Boletus variegatus* and *Clitopilus prunulus*, the former a mycorrhizal fungus with pine, the latter with pine and spruce. These species require only thiamin (vitamin B_1) to be able to develop in a synthetic medium. In the absence of this vitamin no growth takes place.

Most of the mycorrhizal fungi of trees have considerably more complex requirements of vitamins or other growth substances. By way of example I will merely mention *Lactarius deliciosus*. On a synthetic medium containing thiamin its growth is slow, but after addition of certain extracts, e.g. extract of litter or of fungus sporophores, there is a considerable acceleration in growth (Melin, 1946). Small amounts of extract exercise a very favourable effect. Rather large additions, however, inhibit growth on account of the presence of antibiotic substances. For the present the nature of the vitamins necessary for the growth of this species and of most other mycorrhizal fungi of trees is unknown.

Tree-mycorrhiza fungi all develop vigorously and in a characteristic manner between the cortical cells of the roots after the middle lamellae have decomposed. This suggests that pectins admit of being readily utilized by the fungi. Certain sugars are also good sources of energy. On the other hand, the celluloses and lignins do not seem to be utilized as sole sources of carbon, at any rate in pure culture. Under certain conditions, however, the mycorrhizal fungi would appear to form cellulose-splitting enzymes, which enables them to pierce the cell walls and invade the cells.

The mycorrhizal fungi are able to make use of inorganic as well as organic nitrogenous compounds as sources of nitrogen, as do the litter-decomposing and wood-destroying Basidiomycetes. Proteolytic enzymes, nuclease and amidases have been demonstrated.

It has for long been known that the tree mycorrhiza are at times formed sparingly and with difficulty in nature, even when there are abundant hyphae of suitable mycorrhizal fungi in the soil. In Sweden this is the case in certain pine heaths and in old feebly growing spruce woods with inactive raw humus. In that country well-developed mycorrhiza of pine and spruce

are to be found in mixed forests where there is active raw humus and the processes of decomposition are more or less rapid.

The conditions for the formation of mycorrhiza in seedlings of pine have been thoroughly studied during the last few years by two of my pupils, Dr A. B. Hatch and Dr E. Björkman. Hatch (1937) arrived at the result that mycorrhiza is formed if the soil and hence also the roots are deficient in one or more of the substances nitrogen, phosphorus, potassium, or calcium. According to Björkman (1942), there are mainly three factors that have an important influence on the formation of mycorrhiza of trees, viz. light and the quantities of nitrogen and phosphorus available in the soil. Björkman cultivated pine and spruce seedlings under different intensities of light in different types of humus. In the dark or in weak light up to about 10% of full daylight no mycorrhiza developed as a rule. If the intensity of the illumination was increased from 10 to 25%, however, a powerful increase in mycorrhiza formation resulted. After a further intensification of the light there was but a slight increase.

When there was a severe lack of assimilable nitrogen or phosphorus in the substratum, the formation of tree mycorrhiza was inconsiderable. If cellulose was mixed in forest soil in which mycorrhiza is normally developed with facility, mycorrhiza formation was reduced or stopped. This may be because cellulose-decomposing fungi and bacteria are stimulated to increased activity and rapidly exhaust the soluble nitrogen. Nor was any mycorrhiza developed if the soil contained both nitrogen and phosphorus in large quantities. On the other hand, such was formed optimally if, under favourable conditions in other respects, available nitrogen or phosphorus or both occurred in moderate but suboptimum quantities.

The same results were obtained whether the experiments were carried out under aseptic conditions in pure culture or with natural forest soils to which ammonium nitrate and phosphoric acid had been added in varying amounts. I should like to cite for illustration two series of experiments of the last-mentioned kind. The type of humus used had different properties, one being an inactive raw humus (*K*) that was extremely poor in ammonium nitrogen but rich in phosphoric acid, the other being a peat soil (*H*) rich in ammonium nitrogen but poor in phosphoric acid. In the *K* soil the growth of the pine seedlings was accelerated by ammonium nitrate but not by phosphoric acid. In the *H* soil the reverse condition prevailed: phosphoric acid but not nitrogen stimulated the growth of the plants. On addition of ammonium nitrate the mycorrhiza frequency was reduced in the *K* soil but not in the *H* soil. If phosphoric acid was supplied instead, there was a reduction in the development of mycorrhiza in the *H* soil but not in the *K* soil. On the other hand, a supply of both nitrogen and phosphorus brought about a powerful decline in the mycorrhiza frequency in both cases.

In what, then, does the significance of these mycorrhiza-affecting factors lie? The fact that such diverse factors as those mentioned produce the same effect suggests that the explanation lies in their exercising an indirect influence. It is possible that their significance lies in the fact that they affect the sugar content of the roots and that this, directly or indirectly, has an

influence on the formation of mycorrhiza. Under the highest intensities of light there goes on the most active production of carbohydrates, which among other things are used for the building up of proteins and nucleic acids. If there is a deficiency of nitrogen or phosphorus, the protein synthesis is inhibited and there consequently arises a surplus of soluble carbohydrates in the plant as also in its roots, with the result that the penetration of mycorrhizal fungi into the latter is favoured. Conversely, if both nitrogen and phosphorus are available in abundant quantities, the protein and nucleic-acid syntheses are favoured and no excess of soluble carbohydrates is established. It would accordingly seem that mycorrhiza fails to appear because the fungi then get no source of energy in the roots. Certain experiments with pine seedlings that had been strangulated argue in favour of this view (Björkman, 1944). Three-year-old pines with well-developed mycorrhiza were strangulated during the spring, and the effect of the strangulation on the development of mycorrhiza was studied the following autumn. In the control plants mycorrhiza was well developed, but was almost completely absent in the strangulated plants. It is possible that the obstructed transport of sugar to the roots was the cause of the failure of mycorrhiza to appear. Other explanations are not excluded, however. Against the sugar hypothesis is, first and foremost, McDougall and Dufrenoy's (1944) observation that under certain conditions excised pine mycorrhiza can survive and continue to live in forest soil.

Other factors may also affect the formation of tree mycorrhiza. As long as twenty years ago I pointed out (Melin, 1925) the possibility that substances may at times be formed in the soil which inhibit the growth of the mycorrhizal fungi, and thereby render the formation of tree mycorrhiza more difficult. The questions concerned here have acquired a deeper interest as a result of modern research relating to substances that exercise an antibiotic effect on pathogenic bacteria. It is now an established fact that a large number of fungi and bacteria exude substances that retard or prevent the growth of other micro-organisms. Probably, such substances influence and regulate to a high degree the development of the micro-organisms, including the mycorrhizal fungi in the soil. Rayner and Neilson-Jones (1944) have recently demonstrated that toxic substances obstruct or even prevent mycorrhiza formation in a moorland area in southern England, viz. Wareham Forest, Dorset. In forest soil, too, substances may occur that inhibit the growth of the mycorrhizal fungi. Under what conditions they are formed and what inhibitory influence they have on the formation of mycorrhiza still remains to be elucidated.

I have recently demonstrated that the leaves and needles which are shed during the autumn in the Swedish forests contain water-soluble, thermo-stable substances that exercise a strong antibiotic effect on tree-mycorrhiza fungi. This is the case with leaf litter of maple, birch, beech, oak, aspen and pine. The experimental method employed was to extract the ground-up leaves with water, whereupon the extracts were autoclaved or passed through a Seitz filter. In low concentration the extracts had a stimulating effect, whereas in higher concentration they prevented the growth of the mycorrhizal fungi (Melin, 1946).

The presence of inhibitory substances is the reason that the mycorrhizal fungi do not thrive and develop in forest litter. In course of time, however, these substances disappear as a result of washing-out or decomposition. Probably, therefore, any antibiotic substances present in forest humus have been newly formed by micro-organisms in the soil.

The question of the importance of mycorrhiza for trees has been lately studied by A. B. Hatch (1936, 1937). As a substratum Hatch used prairie soil from a forestless tract in Wyoming, U.S.A. In August 1934, seedlings of *Pinus strobus* were planted in test jars containing the humus mentioned. Half of them were inoculated with pure cultures of known mycorrhizal fungi, among others *Lactarius deliciosus*. The uninoculated plants developed feebly and soon assumed a yellowish tint, while those inoculated grew vigorously and developed dark-green needles. The experiment was broken off in May 1935. The inoculated plants then had well-developed mycorrhiza, whereas the feeble plants were entirely destitute of such. Chemical analyses were undertaken and showed that the mycorrhiza-bearing plants had a much higher nitrogen content than the control plants. In addition, the former had double as high contents of phosphorus and potassium as the controls. Later experiments with different kinds of trees have confirmed these results.

By these investigations it has been conclusively proved that tree mycorrhiza as a nutriment-absorbing organ, under certain conditions, is more effective than the roots alone. In forest soils having a deficiency of nitrogen, phosphorus or of both these substances, i.e. under favourable conditions for the development of mycorrhiza, the mycorrhizal fungi can compete more easily than the roots with soil micro-organisms for nourishment. The value of symbiosis to both the partners therefore seems to be cleared up on the whole: the fungi obtain carbohydrates from the roots and in addition the necessary vitamins, while the roots obtain nitrogen and other substances essential to life by way of the fungi. There is much to suggest, however, that different species of mycorrhizal fungi are not equally effective in their contribution to the nourishment of the trees. Different mycorrhizal combinations are therefore probably not physiologically equivalent, and generalizations should be avoided. It will be an important task for future research to compare, from a tropho-physiological standpoint, various combinations of mycorrhiza on one and the same tree.

REFERENCES

Björkman, E. (1942). Über die Bedingungen der Mykorrhizabildung bei Kiefer und Fichte. *Symb. Bot. Upsal.* VI, no. 2.

Björkman, E. (1944). The effect of strangulation on the formation of mycorrhiza in pine. *Svensk Bot. Tidskr.* XXXVIII.

Buller, A. H. R. (1931). *Researches on Fungi*, vol. IV, London.

Clements, J. B. (1938). Trials in Nyasaland of introduced pines. *Australian Forestry*, III.

Cromer, D. A. N. (1935). The significance of the mycorrhizae of *Pinus radiata*. *Commonwealth For. Bur. Bull.* no. 16, Canberra, Australia.

Doak, K. D. (1934). Fungi that produce ectotrophic mycorrhizae of conifers. *Phytopathology*, XXIV.

Ferdinandsen, C. & Winge, Ø. (1925). *Cenococcum* Fr. A monographic study. *Kgl. Veterinaer- og Landbohøjskole, Aarsskrift, København.*

HATCH, A. B. (1934). A jet black mycelium forming ectotrophic mycorrhizae. *Svensk Bot. Tidskr.* XXVIII.

HATCH, A. B. (1936). The role of mycorrhizae in afforestation. *Jour. For.* XXXIV.

HATCH, A. B. (1937). The physical basis of mycotrophy in *Pinus. Black Rock. For. Bull.*, no. 6.

HATCH, A. B. & HATCH, C. T. (1933). Some Hymenomycetes forming ectotrophic mycorrhizae with *Pinus Strobus* L. *J. Arnold Arb.* XIV.

KESSEL, S. L. (1927). Soil Organisms. The dependence of certain pine species on a biological soil factor. *Empire For. Journal*, VI.

LIHNELL, D. (1942). *Cenococcum graniforme* als Mykorrhizabildner von Waldbäumen. *Symb. Bot. Upsal.* V, no. 2.

LINDEBERG, G. (1944). Über die Physiologie ligninabbauender Bodenhymenomyzeten. *Symb. Bot. Upsal.* VIII, no. 2.

MCDOUGAL, D. T. & DUFRENOY, J. (1944). Mycorrhizal symbiosis in *Aplectrum, Corallorhiza* and *Pinus*. *Plant Physiol.* XIX.

MELIN, E. (1917). Studier över der norrländska myrmarkernas vegetation. *Norrländskt Handbibliotek*, VII.

MELIN, E. (1921). Über die Mykorrhizenpilze von *Pinus silvestris* und *Picea Abies*. *Svensk Bot. Tidskr.* XV.

MELIN, E. (1922). Untersuchungen über die *Larix*-mykorrhiza. I. Synthese der Mykorrhiza in Reinkultur. *Svensk Bot. Tidskr.* XVI.

MELIN, E. (1923*a*). Experimentelle Untersuchungen über die Konstitution und Ökologie der Mykorrhizen von *Pinus silvestris* und *Picea Abies*. *Mykol. Unters. u. Ber. von R. Flack*, II.

MELIN, E. (1923*b*). Experimentelle Untersuchungen über die Birken- und Espenmykorrhizen und ihre Pilzsymbionten. *Svensk Bot. Tidskr.* XVII.

MELIN, E. (1925). *Untersuchungen über die Bedeutung der Baummykorrhiza. Eine ökologisch-physiologische Studie.* Jena.

MELIN, E. (1936). Methoden der experimentellen Untersuchung mykotropher Pflanzen. *Handb. biol. Arbeitsmethoden*, XI, no. 4.

MELIN, E. (1946). Der Einfluss von Waldstreuextrakten auf das Wachstum von Bodenpilzen, mit besonderer Berücksichtigung der Wurzelpilze von Bäumen. *Symb. Bot. Upsal.* VIII, 3.

MELIN, E. & LINDEBERG, G. (1939). Über den Einfluss von Aneurin und Biotin auf das Wachstum einiger Mykorrhizenpilze. *Bot. Notiser.*

MELIN, E. & NORKRANS, B. (1942). Über den Einfluss der Pyrimidin- und der Thiazol-Komponente des Aneurins auf das Wachstum von Wurzelpilzen. *Svensk Bot. Tidskr.* XXXVI.

MELIN, E. & NYMAN, B. (1940). Weitere Untersuchungen über die Wirkung von Aneurin und Biotin auf das Wachstum von Wurzelpilzen. *Arch. Mikrobiol.* XI.

MODESS, O. (1941). Zur Kenntnis der Mykorrhizabildner von Kiefer und Fichte. *Symb. Bot. Upsal.* V, no. 1.

OLIVEROS, S. (1932). Effect of soil inoculation on the growth of Benquet pine. *Makiling Echo, Bur. of Forestry, Dept. of Agr. and natural resources of the Philippine Islands*, XI.

RAYNER, M. C. & NEILSON-JONES, W. (1944). *Problems in tree nutrition.* London.

WHITE, D. P. (1941). Prairie soil as a medium for tree growth. *Ecology*, XXII.

A CONTRIBUTION TO THE STUDY OF FUNGI IN THE SOIL

By CHARLES G. C. CHESTERS
Department of Botany, University College, Nottingham

(With 5 Text-figures)

No single method of examining the soil microflora gives more than an approximate picture of the fungal population and specially of the actively growing fungi present in the soil at any instant of time. Little is known of the particular habitats of the common soil saprophytes, of the exact periods of their active growth and of the succession of species which may occur on organic debris in the soil. The method of investigation described in the following pages was devised with the idea of supplying an initial approach to these problems. It consists of removing a soil sample with a borer and placing in the hole thus made an immersion tube (Chesters, 1940) containing sterile nutrient agar. The organic debris in the soil sample is removed from aqueous suspension on screens of different pore size and is there thoroughly washed for subsequent examination and for the isolation of the fungi it contains.

The routine employed consists of:

(1) Removal of a core of soil by means of an alcohol-sterilized borer.

(2) Inserting in the hole, so formed, an immersion tube containing sterile nutrient agar. This tube remains in position for seven days and is then removed for the isolation of the fungi it contains.

(3) Preparation of a suspension in water from the core of soil and separation of the organic debris in this soil on screens of different pore size during a period of washing.

(4) Isolation of the fungi in the suspension and in the debris.

Immersion tubes

Six years ago a brief outline was given of the immersion-tube method of isolating fungi from the soil (Chesters, 1940); the method has been used since to examine the fungal content of soils in the Midlands and elsewhere. The original method has been improved and standardized to allow rapid handling of numerous isolations.

The immersion tubes at present in use are prepared from 6 inches by $\frac{3}{4}$ inch hard glass test-tubes. After tapering the lower inch of the tube, and while the glass is still warm, four or six capillaries are drawn out from the wall to form a spiral series, commencing about one and a half inches from the bottom of the tube and ending three inches from the bottom of the tube. These capillaries are then cut short, fused and invaginated by means of a warm, waxed needle. The series must be rapidly completed and the finished tube must be thoroughly annealed. When cool, the capillaries form

short, closed cones of glass. Their apices are broken by a sharp file-like needle to give a terminal pore of about 1·5 mm. in diameter and, in so doing, the capillaries are shortened to such a length that a rod, one quarter of an inch in diameter, standing vertically in the tube does not touch any capillary. Each tube which passes this test is numbered.

The tubes can be filled with agar medium or any other solid substrate suitable for the growth of fungi. In routine use the immersion tubes are filled with soil extract agar; the extract being prepared as directed by Lohnis (1913), with the addition of yeast extract, glucose and potassium

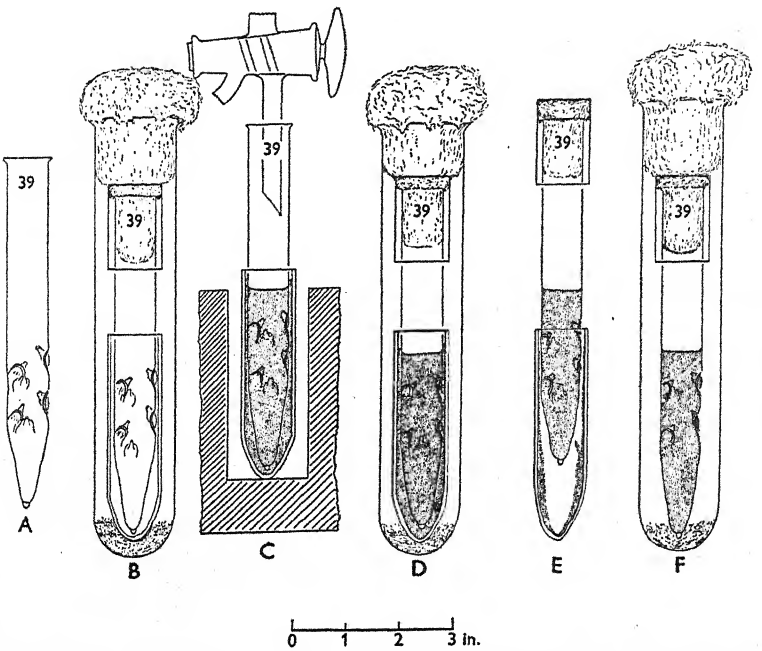


Fig. 1. A, standard type immersion tube with six capillaries; B, plugged immersion tube with jacket and cap, enclosed in boiling tube for sterilization; C, filling immersion tube with agar medium while enclosed in jacket; D, final sterilization when filled with agar; E, removing the jacket and the agar external to the capillaries; F, the filled immersion tube ready for transport to the field.

dihydrogen phosphate, as suggested by Timonin (1940). The tubes are prepared for filling by enclosing the portion carrying the capillaries in a jacket tube of slightly larger diameter (Fig. 1). The plug of the immersion tube is covered by a small specimen tube which later acts as a protection against rain when the tube is in the soil. The complete unit is enclosed in a plugged boiling tube and sterilized. When cool, the jacketed immersion tube is removed from its container and placed in a stand and a measured quantity of sterile melted agar is introduced from a filling burette. This should be sufficient to fill the immersion tube to not less than half an inch above the inner limit of the highest capillary and to allow for overflow

through the capillaries into the space between the tube and the jacket. When the agar has set the plugged and capped immersion tube is returned to its container, resterilized and set aside to cool. Under a transfer hood the immersion tube is pulled from the jacket tube leaving much of the overflow agar behind. Any that remains in the outer openings of the capillaries is excised with a flamed needle, and the whole tube surface is swabbed with alcohol and flamed dry. Replaced in its container the immersion tube is carried into the field for insertion in the soil.

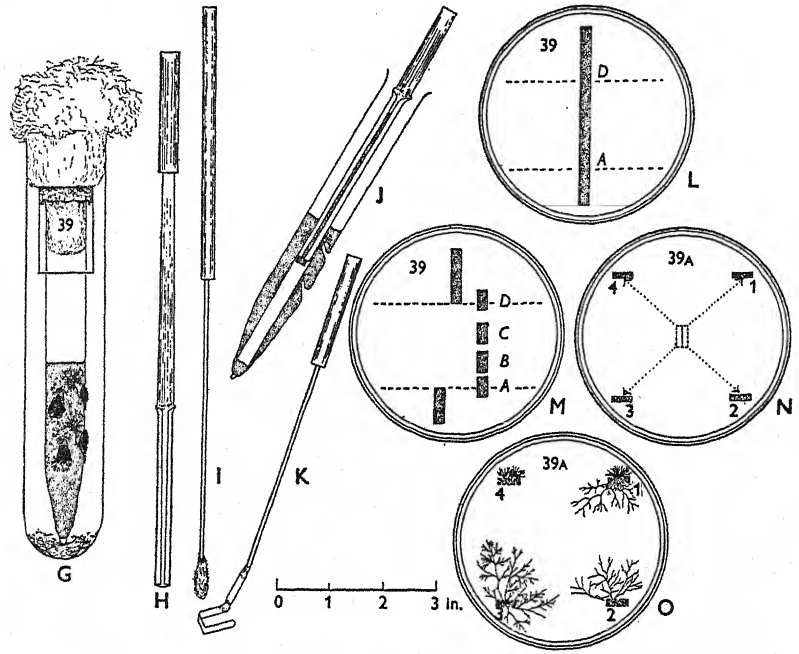


Fig. 2. G, immersion tube as it is extracted from the soil after incubation for 7 days; H, stainless steel corer for extracting the agar inoculum; I, swab for extrusion of inoculum core; J, process of removing inoculum core from agar of immersion tube; K, knife used to cut core (L) into equal cylinders within the area occupied by capillaries; M, the separation and labelling of the cylindrical inocula from a core; N, the preparation of four equal sectors from one cylindrical inoculum and their disposition before covering with the sterile agar; O, similar plate after period of incubation, showing growth from the sectors of the original cylinder.

Prepared immersion tubes can be exposed in the laboratory for at least two days without showing signs of contamination when incubated in a sterile container.

In the field the immersion tube is placed in firm soil or turf by removing a core of soil of slightly less diameter than the tube by means of a flame-sterilized coring iron. The tube is inserted immediately the core is withdrawn and then fits tightly against the soil. Incubation for seven days provides adequate growth of mycelium inside the agar (Fig. 2). Removed to the laboratory, a core of agar one-quarter of an inch in diameter is cut from the length of the agar by a stainless steel corer. This core is extruded into

a Petri dish by means of a sterile swab. The limits of the upper and lower capillaries are marked on the dish and, between these marks, four equal cylinders are excised with a U-shaped knife. Each cylinder is removed to a separate dish and is there cut longitudinally into four equal sectors which are placed at equal distances round the periphery of the dish. When a batch of tubes has been dealt with in this way sterile agar medium, cooled to 50° C., is poured into the centre of the first prepared Petri dish and is allowed to flow evenly over the dish. This does not disturb the agar sectors which, during the waiting period, have become lightly fixed to the glass. Control tubes, which have not been in contact with the soil, seldom show fungal growth and indicate freedom from contamination during the isolation process. Fungi which develop on inoculated dishes can be referred to the segments of the core from which they were obtained and results are normally recorded to indicate the origin of colonies in relation to the core segments (Fig. 3).

Experiments using agar media have shown that in cultivated and pasture soils the dominant fungi can be arranged in the following series, which indicates the relative frequency of the genera isolated:

(1) *Mortierella, Mucor, Pythium.*

(2) *Rhizoctonia, Rhizopus, Zygorhynchus, Fusarium.*

(3) *Cephalosporium, Circinella, Cunninghamella, Sordaria, Hyalopus, Botrytis, Phoma.*

In addition, numerous sterile mycelia are isolated which usually fail to develop spores in normal agar culture. Very occasionally species of *Penicillium* are isolated, and even less frequently *Armillaria mellea.* The precise fungi isolated in any one experiment depend upon the soil investigated and the medium used for isolation. Two examples will illustrate this point. Using maize-extract agar and isolating from the 'Abbey' soils of Evesham the following list indicates the range commonly obtained in descending order of dominance:

Pythium intermedium	*Mortierella* sp.
Pythium ultimum	*Rhizoctonia solani*
Fusarium sp.	

Using 2% malt-extract agar in the same soils the following series was obtained:

Mucor sp.	*Fusarium* sp.
Rhizoctonia solani	

Isolations from old pasture turf over Bunter sandstone soil on maize-extract agar provided:

Mortierella sp.	*Pythium artotrogus*
Pythium intermedium	*Rhizoctonia solani*
Pythium ultimum	*Mucor* sp.
Pythium mamillatum	

In the same soil the list for 2% malt-extract agar provided:

Mucor sp.	*Fusarium* sp.
Rhizoctonia solani	*Mortierella* sp.

Certain features are worthy of comment. Species of *Pythium* occur only in the maize-extract agar series and such species can be constantly isolated from soils with an adequate humus content by employing this medium.

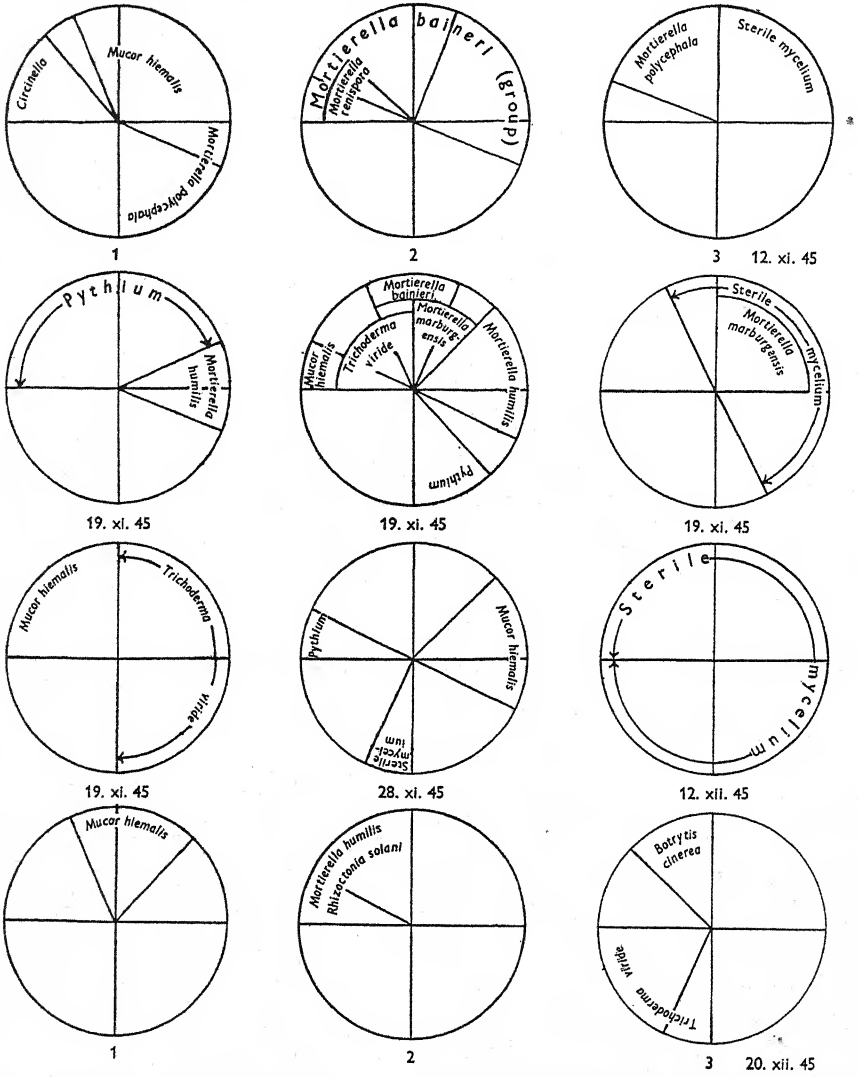


Fig. 3. Twelve charts illustrating the method of recording results from immersion-tube isolations. Each quarter of a circle represents the isolations from a single cylinder of agar (Fig. 2 M, *A–D*). Where isolations vary in each sector of such a cylinder, the species isolated are indicated in the appropriate sectors of each quarter of a circle.

Although species of *Mucor* and related genera do appear in maize-extract agar, they are more easily obtained on malt-extract agar, a characteristic which also applies to species of *Fusarium* and to *Rhizoctonia solani*. Soil-

extract agar permits a more even distribution of the species in immersion tubes, but there is a decided tendency to limit the growth of species of *Pythium*. The addition of plant extracts or of prepared nutrient extracts to soil agar favours the development of those species appearing predominantly in the particular extract. *Trichoderma viride* and species of *Penicillium* occur infrequently in routine isolations from immersion tubes, even when they are abundantly represented in plated suspensions of the same soil made at the time of isolation from the tubes. So far as *Trichoderma viride* is concerned the sporadic appearance is not due to its inability to infect immersion tubes, as is shown by the following experiment.

Four equal quantities of a sample of sterilized compost consisting of equal parts of soil, leaf-mould and sand were inoculated respectively with equally dense spore suspensions of *T. viride*, *Mucor hiemalis* and *Mortierella renispora* and with mycelial fragments of *Pythium ultimum*. Two days after inoculation and again seven days after inoculation, immersion tubes were introduced into the composts. The experiment was conducted out-of-doors and, during the *Mucor* and *Trichoderma* experiments, bright dry weather predominated whereas, during the *Mortierella* and *Pythium* experiments, heavy rain waterlogged the compost and caused considerable contamination. The results of this experiment are set out in Table 1.

The composts were examined for the presence of mycelium and spores at the end of seven days and fourteen days. It was difficult to distinguish between the *Pythium* mycelium and the contaminating *Mucor* in the *Pythium* series. In the *Mucor* series, widespread sporangial-bearing mycelium was visible after seven days, and in the *Trichoderma* series spreading mycelium, causing aggregation of the soil particles, was seen after seven days and sporing clusters were seen after fourteen days.

The experiment demonstrates that when active, widespread mycelium of *Trichoderma viride* is present in soil it can be recovered to almost 100% by the immersion-tube method in the absence of competition from other fungal species. Its failure to appear in routine isolations may be due to factors restricting its spread in the soil to its immediate medium or to competition of faster growing species in the capillaries of the immersion tubes, or to the fact that vigorous sporulation in the soil causes early cessation of vegetative growth. Our view of the limits of the immersion-tube method is that it easily isolates active spreading mycelium or active localized mycelium which happens to come into contact with the capillaries. The fungi so isolated must obviously be capable of growth into and through the medium in the tube, and preliminary experiments indicate that a control of the isolated fungi may be effected at this point.

To explore this view further an attempt was made to separate plant debris from the soil and to determine the relationship of fungal species to this debris. Finally, parallel isolations from this debris were compared with plated suspensions and with immersion-tube isolations. This work was carried out on pasture turf overlying a Keuper soil.

Extract agar from this soil was used throughout the experiments. Twelve selected immersion-tube isolations, displayed in Fig. 3, indicate the range of species isolated during November and December 1945.

Table 1

Tubes inserted two days after inoculation. Removed nine days after inoculation

Soil inoculated with:	Control	1	2	3	4	Level
Mortierella renispora Dixon-Stewart	*B.*, *M.h.* (2)	2 *M.h.* (1)	4 *M.h.* (2)	1	3 *M.h.* (2)	A
	B., *M.h.* (1)	4	2 *M.h.* (2)	2	2 *M.h.* (1)	B
	B.	3	2 *M.h.* (1)	3	4	C
	B.	3	—	4	4	D
Pythium ultimum Trow	*R.*	2	Bacteria	Bacteria	Bacteria	A
	B.	4	Bacteria	Bacteria	Bacteria	B
	B.	1	Bacteria	Bacteria	Bacteria	C
	B.	—	Bacteria	Bacteria	Bacteria	D
Mucor hiemalis Wehm.	—	4	3	2	4	A
	—	4	2	4	1	B
	—	3	3	4	4	C
	—	1	1	4	4	D
Trichoderma viride Fr.	—	4	3	1	4	A
	—	4	4	2	4	B
	—	3	3	—	2	C
	—	3	3	1	2	D

Tubes inserted seven days after inoculation. Removed fourteen days after inoculation

Soil inoculated with:	Control	1	2	3	4	Level
Mortierella renispora Dixon-Stewart	*B.*, *M.h.*	—	—	3	4	A
	B., *M.h.*	1	4	4	2	B
	B., *M.h.*	3	3	3	4	C
	M.h.	3	—	3	3	D
Pythium ultimum Trow	*B.*, *M.h.*	*M.h.* (4)	4	2	4	A
	B., *M.h.*	*M.h.* (4)	2	3 *M.h.* (1)	4	B
	B., *M.h.*	1 *M.h.* (3)	2	1 *M.h.* (4)	4	C
	B., *M.h.*	2	—	1 *M.h.* (1)	4	D
Mucor hiemalis Wehm.	*B.*, *P.*	4	4	4	4	A
	B., *P.*	4	4	3	4	B
	B.	4	4	2	2	C
	B.	4	4	4	3	D
Trichoderma viride Fr.	*B.*	4	4	1	4	A
	B.	4	4	4	4	B
	B.	4	4	4	4	C
	B.	4	4	3	4	D

B. = bacteria. *M.h.* = *Mucor hiemalis* Wehm.
P. = *Pyronema confluens* (Pers.) Tul. *R.* = *Rhizoctonia solani* Kühn.

Each figure represents the contents of a complete tube in which the individual quadrants represent the four cylinders cut from an immersion-tube core. The four smaller quadrants in each larger quadrant represent the individual sectors of each cylinder. Each diagram is so orientated that, passing from the north point by east, south and west the major quadrants pass downwards from the highest capillary to the lowest. These results may be summarized as follows:

Fungi isolated	Sectors involved	Fungi isolated	Sectors involved
Sterile mycelia	7	*Pythium* (*intermedium*)	2·5
**Mortierella* sp.	5	*Botrytis cinerea*	0·5
Mucor sp.	4	*Circinella* sp.	0·5
Trichoderma viride	3·75		

* In this experiment and elsewhere in this paper species names refer to the nearest 'group' into which the isolation can be fitted and do not indicate final specific identification. Detailed studies on the *Mortierella* species in soil are in progress.

The separation of organic debris from soil

Simultaneously, experiments were in progress to devise means of separating organic debris from the mineral matter of the turf by dispersing the debris in water, washing it free of mineral fragments on sterile screens, and finally thoroughly washing it in sterile water. Without entering into details of these initial trials Table 2 shows the species isolated from three cores of the turf soil between November 1945 and February 1946. Only root fragments

Table 2

Species isolated from root debris in the 'turf' layer	Sample 11	13	15
Absidia cylindrospora Hag.	2	4	1
Absidia glauca Hag.	2	—	—
Mortierella bainieri Cost.	—	3	—
Mortierella canina Dauph.	—	—	1
Mortierella humilis Linneman	1	6	2
Mortierella simplex v. Tiegh. & Le Mon.	—	—	1
Mucor hiemalis Wehm.	5	4	3
Mucor ramannianus Möll.	1	3	—
Zygorhynchus moelleri Vuill.	—	1	—
Helminthosporium biforme Mason & Hughes n.sp.	—	1	1
Cephalosporium asperum Marchal	—	—	1
Species of *Fusarium*	—	—	3
Species of *Penicillium*	2	5	4
Trichoderma viride Fr.	6	—	4

were considered and only mycelia growing from within such fragments after a minimum incubation period were isolated. Of the species frequently isolated:

Mucor hiemalis	occurred	on	12	fragments
Trichoderma viride	,,	,,	10	,,
Mortierella humilis	,,	,,	9	,,
Absidia cylindrospora	,,	,,	7	,,
Penicillium sp.	,,	,,	11	,,

The species listed as *Helminthosporium biforme* Mason & Hughes n.sp., and which is described by these authors in a specially written appendix to this paper, was obtained from two root fragments, but it recurs with some regularity on root debris from this soil. I am deeply indebted to Mr Mason and Mr Hughes for their description, diagnosis, discussion and illustration of this species. It may be the same fungus as that illustrated by Kubienna & Renn (1935) as *Acrothecium* species, from a somewhat similar habitat.

The initial experiments having demonstrated the possibility of obtaining washed debris by aseptic transfer from a soil sample, a more efficient means of handling the sample was devised. The apparatus consisted of three units, sterilized separately and assembled with quick-fit, metal unions (Fig. 4). The first unit was a two-litre reservoir for sterile distilled water carrying an air filter attached to the inlet end of a metal screw top and a length of pressure tubing attached to the exit end, its free extremity carrying one part of a metal union. The second unit consisted of a screening tube with a coarse mesh screen fixed by a copper ring in the middle of its

length and a fine mesh screen inserted in the lower bung of the tube. The upper bung carried an air filter and a length of rubber tubing ending in one part of a metal union. Below the fine mesh screen an inverted Y-tube permitted the washing water to pass either into a sample tube (*C*) held under a dust bell or into the third unit, which consisted of a two-litre reservoir. The inlet of the screw top carried a length of pressure tubing

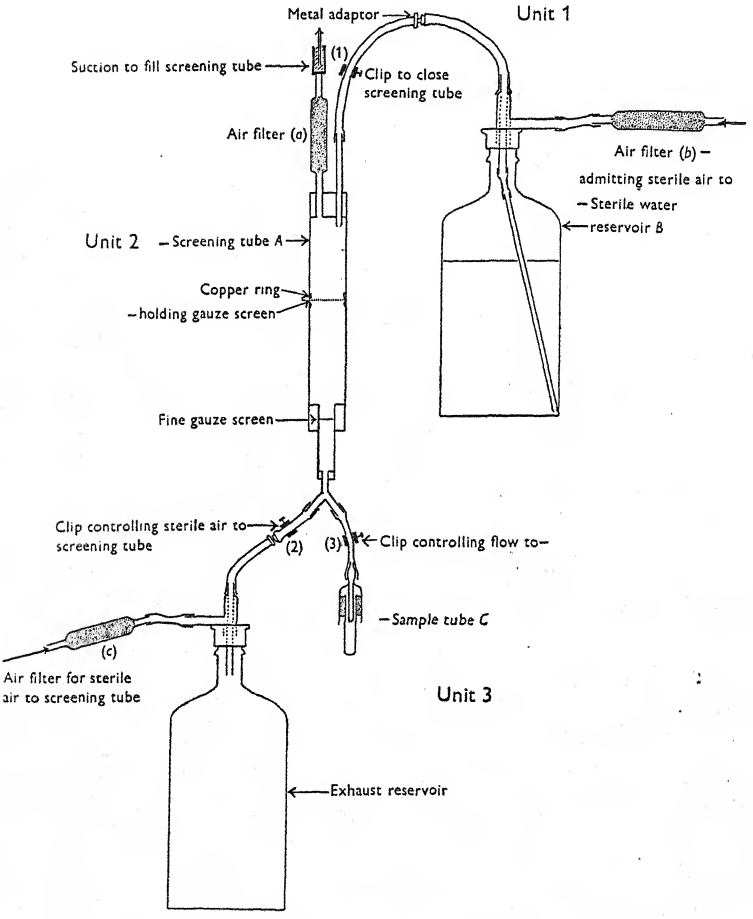


Fig. 4. For detailed explanation see text.

ending in a metal union and the exit carried an air filter. All joints in the apparatus were lagged with cotton-wool and covered with gauze and medical tape, and the open ends of metal unions and air filters were wrapped in cotton-wool covered with non-absorbent cellophane during sterilization.

The soil sample to be washed is obtained from the desired level by a flame-sterilized corer and is transferred to the laboratory in a sealed tube. It is there removed to a sterile bottle containing 50 ml. water and ten glass

beads. The several bottles of one experiment are shaken in a rotary shaker for thirty minutes. The contents of a bottle are then decanted into the screening tube—clips (2) and (3) being closed (Fig. 4). This unit is then attached to units one and three and is charged with water from reservoir (*B*) by suction at filter (*a*)—clips (2) and (3) still remaining closed. The volume of water should be sufficient to provide washing without danger of wetting the upper bung when the mixture is aerated. The water flow is cut off by closing clip (1). Still maintaining suction at filter (*a*), clip (2) is opened and a stream of sterile air is drawn upwards through the mixture from filter (*c*). This aeration continues for ten minutes after which the suspension is drained through the screens to the Y-tube, leaving the organic debris on the filters. A measured quantity of the suspension is obtained in sample tube (*C*) and the remainder is drained into the exhaust reservoir. This washing process is repeated until about two litres of water have passed through the screening tube and the debris is free of all mineral matter. The debris is then removed from each screen to 100 ml. sterile soil-extract agar cooled to 50° C., distributed by shaking, and equal quantities poured into five Petri dishes. After incubation, the fungi growing from the particles of debris are isolated, and careful notes of the kind and state of the particles are made.

Tables 3–5 show the results obtained from such isolated debris and from the suspension of the first washing diluted before being plated. In these experiments isolations were made from the turf *in situ* and from turf which had been buried for a varying length of time.

Table 3

Species isolated from debris in the 'turf' layer	Surface				Buried 16 days				Buried 31 days			
	L.	S.	R.	O.D.	L.	S.	R.	O.D.	L.	S.	R.	O.D.
Absidia glauca Hag.	—	—	—	1	1	—	—	—	—	—	—	—
Absidia orchidis (Vuill.) Hag.	—	—	—	—	—	1	—	—	—	1	—	—
Mortierella simplex v. Tiegh. & Le Mon.	—	—	—	—	—	—	—	—	1	2	—	—
Mucor hiemalis Wehm.	1	1	3	2	5	9	1	—	4	1	—	3
Mucor ramannianus Möll.	1	—	—	1	—	—	2	1	1	1	2	1
Zygorhynchus moelleri Vuill.	—	—	—	—	—	—	—	—	1	—	2	—
Helminthosporium biforme Mason & Hughes	—	—	—	—	—	—	—	—	—	—	1	—
Species of *Penicillium*	2	—	2	2	—	—	—	—	—	1	—	1
Trichoderma viride Fr.	—	1	—	—	—	4	3	1	3	3	3	2

L. = grass leaves. S. = grass stems. R. = roots. O.D. = other debris.

Table 4

Species isolated from suspensions from the 'turf' layer	Surface	Buried 16 days	Buried 31 days
Absidia cylindrospora Hag.	1	—	—
Absidia glauca Hag.	1	—	—
Mortierella bainieri Cost.	—	—	1
Mortierella humilis Linneman	—	—	2
Mucor hiemalis Wehm.	1	18	4
Zygorhynchus moelleri Vuill.	—	6	—
Acrostalagmus albus Preuss	1	—	—
Cephalosporium acremonium Corda	—	—	1
Cladosporium herbarum Fr.	1	—	2
Paecilomyces elegans (Corda) ? Thom.	3	—	—
Species of *Penicillium*	17	—	1
Trichoderma viride Fr.	—	2	1

Table 5

Species isolated from debris and suspensions from the 'turf' layer	Surface		Buried 16 days		Buried 31 days	
	D.	S.	D.	S.	D.	S.
Absidia cylindrospora Hag.	—	1	—	—	—	—
Absidia glauca Hag.	1	1	1	—	—	—
Absidia orchidis (Vuill.) Hag.	—	—	1	—	1	—
Mortierella bainieri Cost.	—	—	—	—	—	1
Mortierella humilis Linneman	—	—	—	—	—	2
Mortierella simplex v. Tiegh. & Le Mon.	—	—	—	—	3	—
Mucor hiemalis Wehm.	7	1	15	18	8	4
Mucor ramannianus Möll.	2	—	3	—	5	—
Zygorhynchus moelleri Vuill.	—	—	—	6	3	—
Cladosporium herbarum Fr.	—	1	—	—	—	2
Species of *Penicillium*	6	17	—	—	2	1
Trichoderma viride Fr.	1	—	8	2	11	1

D. = debris, S. = suspensions.

While it is unsafe to draw definite conclusions from such limited experiments it is evident and precisely what is to be expected, that the suspensions provide a slightly wider range of species than the washed debris. Only *Absidia glauca*, *Mucor hiemalis*, *Zygorhynchus moelleri* and *Trichoderma viride* are certainly common to both methods of isolation. Future experiments may confirm the suggestion that the number of species increases for a time after the turf has been buried—the length of this period and the species of fungi involved being conditioned by the nature and the state of the turf which is buried, and by the available types of spores and mycelia.

In a final series of experiments all three methods were applied to turf *in situ* and to buried turf. The cores removed at the insertion of the immersion tubes were used to provide the debris and the suspensions. The immersion tubes were removed seven days after planting. Some of the results of this series are set out in Tables 6–9, which are so arranged that, reading from the top of the table, the fungi isolated from suspensions precede those isolated, in turn, (2) from immersion tubes, (3) from debris, (4) from suspensions and immersion tubes, (5) from suspensions and debris, (6) from immersion tubes and debris and (7) from all three methods.

Tables 6 and 8 represent the results of isolating from surface turf and Tables 7 and 9 of isolating from turf buried respectively seventy-two and ninety-three days. No generalization can be attempted from these four experiments, but two points of interest must be noted. *Mucor hiemalis* is the only species which is recovered by all three methods of isolation. *Trichoderma viride* is constantly isolated from suspensions and from portions of organic debris, but does not appear in the immersion tubes.

The conclusion we should wish to draw from these studies is that this threefold method of investigating the fungal flora of the soil should be capable of providing fuller information of the biological relationships of soil fungi than methods hitherto available. At this stage in the study of soil fungi it seems to us to be of paramount importance to examine in detail what may be termed the substrate relationship of the soil-inhabiting fungi. This very necessary extension of a principle long recognized by the field mycologist to the microcosm of the soil is still hampered by the lack of

Table 6

	Species isolated from	Suspensions	Immersion-tube levels A	B	C	D	Plant debris: Grass Leaves	Stems	Roots	Other debris
1	*Absidia cylindrospora* Hag.	1	—	—	—	—	—	—	—	—
	Mortierella candelabrum v. Tiegh. & Le Mon.	1	—	—	—	—	—	—	—	—
	Mortierella humilis Linnemann	2	—	—	—	—	—	—	—	—
	Mortierella polycephala Coem.	1	—	—	—	—	—	—	—	—
	Mortierella simplex v. Tiegh. & Le Mon.	1	—	—	—	—	—	—	—	—
	Mucor ramannianus Möll.	2	—	—	—	—	—	—	—	—
	Botrytis cinerea Fr.	1	—	—	—	—	—	—	—	—
	Cladosporium herbarum Fr.	6	—	—	—	—	—	—	—	—
	Species of *Fusarium*	1	—	—	—	—	—	—	—	—
	Penicillium nigricans Bain.	4	—	—	—	—	—	—	—	—
	Penicillium luteum (group)	1	—	—	—	—	—	—	—	—
	Species of *Sporotrichum*	1	—	—	—	—	—	—	—	—
2	*Mucor silvaticus* Hag.	—	—	—	—	+	—	—	—	—
	Pullularia pullulans (de Bary) Berkhout	—	+	+	+	—	—	—	—	—
3	*Paecilomyces elegans* Corda	—	—	—	—	—	—	—	—	Fully decayed debris (1)
4	*Zygorhynchus moelleri* Vuill.	2	—	+	—	—	—	—	—	—
5	*Absidia glauca* Hag.	2	—	—	—	—	—	—	1	—
	Trichoderma viride Fr.	9	—	—	—	—	—	4	5	Beech leaf (2)
7	*Mucor hiemalis* Wehm.	6	+	+	+	+	—	2	7	Beech leaf (2)

Table 7

	Species isolated from	Suspensions	Immersion-tube levels A	B	C	D	Plant debris: Grass Leaves	Stems	Roots	Other debris
1	*Absidia cylindrospora* Hag.	2	—	—	—	—	—	—	—	—
	Mortierella bainieri Cost.	2	—	—	—	—	—	—	—	—
	Mortierella humilis Linneman	6	—	—	—	—	—	—	—	—
	Mortierella simplex v. Tiegh. & Le Mon.	1	—	—	—	—	—	—	—	—
	Cladosporium herbarum Fr.	1	—	—	—	—	—	—	—	—
	Paecilomyces elegans Corda	4	—	—	—	—	—	—	—	—
	Species of *Sporotrichum*	2	—	—	—	—	—	—	—	—
3	*Absidia glauca* Hag.	—	—	—	—	—	1	—	1	—
	Acremoniella atra Sacc.	—	—	—	—	—	—	1	—	—
	Species of *Fusarium*	—	—	—	—	—	—	1	—	—
	Penicillium nigricans Bain.	—	—	—	—	—	—	—	1	—
4	*Zygorhynchus moelleri* Vuill.	3	+	—	—	—	—	—	—	—
	Pullularia pullulans (de Bary) Berkhout	2	—	+	+	+	—	—	—	—
5	*Mortierella simplex* v. Tiegh. & Le Mon.	1	—	—	—	—	—	—	1	Decayed debris (1)
	Mucor ramannianus Möll.	3	—	—	—	—	—	2	2	—
	Trichoderma viride Fr.	7	—	—	—	—	2	7	6	Beech leaf (3). Decayed debris (3)
7	*Mucor hiemalis* Wehm.	1	—	+	+	+	1	1	—	Beech leaf (2)

Table 8

	Species isolated from	Suspensions	Immersion-tube levels A	B	C	D	Plant debris: Grass Leaves	Stems	Roots	Other debris
1	*Cladosporium herbarum* Fr.	6	—	—	—	—	—	—	—	—
2	*Cunninghamella elegans* Lend.	—	+	—	—	—	—	—	—	—
	Mortierella pilulifera v. Tiegh.	—	+	+	+	—	—	—	—	—
	Sordaria (fimicola)	—	—	+	+	+	—	—	—	—
3	*Absidia glauca* Hag.	—	—	—	—	—	—	—	1	—
	Mortierella pusilla Oudem.	—	—	—	—	—	—	1	—	—
	Penicillium nigricans Bain.	—	—	—	—	—	—	—	—	Decayed debris (3)
	Rhizoctonia Solani Kühn	—	—	—	—	—	—	1	—	—
4	*Mucor hiemalis* Wehm.	1	+	—	—	—	—	—	—	—
5	*Mucor ramannianus* Möll.	6	—	—	—	—	—	—	1	Beech leaf (1)
	Zygorhynchus moelleri Vuill.	1	—	—	—	—	—	—	—	Decayed debris (1)
	Trichoderma viride Fr.	5	—	—	—	—	—	2	1	Decayed debris (1)

Table 9

	Species isolated from	Suspensions	Immersion-tube levels A	B	C	D	Plant debris: Grass Leaves	Stems	Roots	Other debris
1	*Helminthosporium biforme* Mason & Hughes	2	—	—	—	—	—	—	—	—
	Species of *Penicillium*	24	—	—	—	—	—	—	—	—
2	Species of *Fusarium*	—	—	+	—	+	—	—	—	—
3	*Absidia cylindrospora* Hag.	—	—	—	—	—	—	—	—	Glume (1)
	Mortierella humilis Linneman	—	—	—	—	—	1	1	—	—
	Cladosporium herbarum Fr.	—	—	—	—	—	—	2	1	—
	Pullularia pullulans (de Bary) Berkhout	—	—	—	—	—	1	—	—	—
	Paecilomyces elegans Corda	—	—	—	—	—	—	3	1	—
	Species of *Penicillium*	—	—	—	—	—	2	2	2	—
	Species of *Phoma*	—	—	—	—	—	—	—	—	Glume (1)
	Rhizoctonia solani Kühn	—	—	—	—	—	1	1	—	—
5	*Mucor ramannianus* Möll.	3	—	—	—	—	—	1	—	Beech leaf (1). Decayed debris (1)
	Trichoderma viride Fr.	5	—	—	—	—	3	10	6	Glumes and other debris (11)
6	*Mucor hiemalis* Wehm.	—	—	—	+	+	—	—	—	Beech leaf (1)

precise knowledge of the taxonomy of many of the soil moulds. This is still, as it has been in the past, a major and fundamental obstacle to be overcome before the biology of the soil-inhabiting saprophytic fungi can be efficiently studied.

The work reported in this paper has been carried out by Miss M. Ellis, Mr J. Webster and the author, and represents the development of a method of attack on the problem of the biology of soil-inhabiting fungi outlined by the author while a member of the staff of the Department of Botany of the University of Birmingham.

REFERENCES

CHESTERS, C. G. C. (1940). A method of isolating soil fungi. *Trans. Brit. mycol. Soc.* XXIV, 352–5.

KUBIENNA, W. & RENN, C. E. (1935). Micropedological studies of the influence of different organic compounds upon the microflora of the soil. *Zbl. Bakt.* (II), XCI, 267–92.

LOHNIS, F. (1913). *Laboratory methods in Agricultural Bacteriology.* Griffin and Co.

TIMONIN, M. I. (1940). The interaction of higher plants and soil micro-organisms. II. The study of the microbial population of the rhizosphere in relation to resistance of plants to soil-borne disease. *Canad. J. Res.* C, XVIII, 444–55.

APPENDIX

Mr Webster's isolate (Herb. I.M.I. No. 4177) was at first identified as *Brachycladium ramosum* Bainier which is characterized by the presence of two sorts of conidiophores, a fact which has hitherto been overlooked; in fact it conforms so closely with Bainier's account that we should not hesitate to employ Bainier's name if it were available. Our nomenclature card index for this group of fungi, however, shows that the name *B. ramosum* Bainier (1908) is a later homonym of *B. ramosum* (Cooke) A. L. Smith (1903). Accordingly, although it was validly and effectively published, Bainier's binomial cannot be legitimately employed. It has also two taxonomic variants. Of these, the first is *Dendryphion* ramosum* (Bain.) Sacc. & Trav. (1910), which is itself a later homonym of *D. ramosum* Cooke (1871), and the second is *Curvularia ramosa* (Bain.) Boedijn (1933) which was included in his '*lunata*-Gruppe'. Of these three names, *C. ramosa* (Bain.) Boed. is the only one which was not illegitimate at its publication under the later homonym rule.

The exact status of the name *C. ramosa* (Bain.) Boed. remains in some doubt. Authority can be found for the view that it may perhaps be considered a legitimate name, ranking for priority from the date 1933, but no living authority has in fact been found who would recommend its *de facto* use.

Bainier's species accordingly requires another name. It is not a typical *Curvularia* because the most characteristic species of that genus form conidia that are abruptly curved, usually at an enlarged cell. It is not a typical *Dendryphion* or *Brachycladium* as both these genera possess macronematous conidiophores which carry a number of short branches near their apex with conidia in chains produced in acropetal chains. On the other hand, if

* Wallroth spelt the name *Dendryphion* (not *Dendryphium*).

the genus *Helminthosporium* is to contain species that produce their conidia in an ear, each formed successively on the growing-point of the conidiophore, then this is a good enough *Helminthosporium*.

In modern mycology the onus of designating its type is placed on each author who proposes a new name; and the question arises whether we should follow the traditional course of proposing a new name for an old species, in which case the type of the name would be the material from which Bainier drew up his original description of '*B. ramosa*'; or whether we should adopt the alternative of proposing a new name for a new species based upon its own type, in this case our dried cultures of Mr Webster's fungus. Bainier's fungus was found on dead stems of *Urtica dioica* in France, and, as far as is known, it has not since been recognized in its *locus natalis*, nor indeed in Europe. Bainier did not normally keep type specimens of his species, so that to our knowledge no type material of this species is in existence. It appears then that until a fungus agreeing perfectly with that figured by Bainier is re-collected on nettle stems it seems best not to use his type for a new name; such a step would, if based upon a misidentification, only serve to confuse the nomenclature of this species. We consider it nomenclaturally sounder to describe this isolate as new, especially as it is a characteristic inhabitant of roots in turf in the Nottingham district. Possible distinctions between the Webster and Bainier fungi are indicated after the diagnosis.

So long as no one can find any ground for separating the old Bainier collection specifically from the new Webster collection, one name will do for both. If in the unforeseeable future our grouping should have to be divided, it is better that the name *Helminthosporium biforme* should be attached to the Webster collection from which in fact this description was drawn up.

Helminthosporium biforme Mason & Hughes sp.nov.

? syn. *Brachycladium ramosum* Bainier. *Bull. Soc. myc. Fr.* XXIV, p. 80, 1908. non *B. ramosum* (Cooke) A. L. Smith (1903).
Dendryphion ramosum (Bainier) Sacc. & Trav. *Syll. Fungorum*, XIX, p. 559, 1910: non *D. ramosum* Cooke (1871).
Curvularia ramosa (Bainier) Boedijn. *Bull. du Jardin Botan. de Buitenzorg*, Ser. III, 13, Livr. 1, p. 129, 1933.

Coloniae in primo hyalinae floccosaeque, dein in partibus 'mouse-grey 8″″″ i Ridgeway'; a tergo nigrae.

Mycelium ex hyphis immersis in primo hyalinis dein brunneis, 2·5 ad 8·0 μ latis, demum tuberculas substromaticas, planas deinde subcylindricas, basi 200 ad 350 μ latas, ad 500 μ altas efformantibus; et ex hyphis aeris aliquanto tenuioribus constans.

Conidiophora micronematica basi hyphis mycelicis similia, erecta vel decumbentia, 5 ad 6·5 μ lata, apicem versus latiora, brunnescentia, geniculata, auriculam conidiorum densam vel laxam gerentia.

Conidiophora macronematica divergentia ex tuberculis substromaticis oriunda, parte sterili et parte fertili sistentia; pars sterilis recta, subulata nigrobrunnea, crasso-tunicata, 360 μ ad 700 μ longa, basi 10 ad 14 μ, apice

7 ad 8μ lata; pars fertilis apicalis pallidior, ad 70μ longa, auriculam conidiorum singulam gerens.

Conidia typice obovata, aliquando elliptica, recta, aeque colorata, primo subhyalina, dein pallide-brunnea vel brunnea, aeque cellulata, 3 ad 5 plerumque 4, 22 ad 36 × 10 ad 14·5μ; cum exosporio (pellicula) primo subhyalino dein pallide-brunneo vel brunneo et endosporio hyalino quasi sub-gelatinoso 2 ad 3μ lato praedita.

Hab. ex radicum detrito sub caespite gramineo isolatum et in agaro 'Quaker oat' culturum; Nottingham, Angliae. Pars exsiccata in Herb. I.M.I. No. 4177 est typus.

The following description is based upon Mr Webster's isolate when it was grown on Quaker Oat agar; at first it produced its conidia on micronematous conidiophores (Fig. 5, A, B, C) and later, on macronematous conidiophores as well (Fig. 5, D, E).

Immersed hyphae are 2·5–8μ wide and near the surface they often tend to unite into strands. The hyphae are hyaline at first, but become brown in old cultures.

Micronematous conidiophores arise as branches from the superficial hyphae, or the ends of aerial hyphae may broaden, become brown and bear conidia. At first the micronematous conidiophores, which are 5–6·5μ wide, are short and may bear a dry ear of conidia when only about 50μ long. They elongate, however, and branch, producing conidia densely or sparsely along their whole length or only at intervals along their length. A 'mouse grey 8″″ i' (Ridgeway) floccose mat of micronematous conidiophores is thus formed and this reaches a height of about 1 mm.

Conidia are produced terminally and a subterminal growing-point arises which turns the conidium to one side; another terminal conidium is formed and by the production of a succession of growing-points the process is repeated. The conidia are generally obovate, sometimes elliptical, evenly pale brown to brown with a well-marked more darkly coloured scar. Conidia are provided with a pellicle or exosporium which encloses 3–5, mostly 4 equal cells each of which is provided with a thick endosporium 2–3μ wide. Fifty conidia from micronematous conidiophores measured 22–36 by 10–14·5μ with a mean of 29 by 13μ.

At scattered points on the agar groups of hyphae with swollen, almost barrel-shaped cells, unite to form a pseudoparenchymatous elevation. The cells composing this stroma are almost globose and become very dark brown; from some of the surface cells the macronematous conidiophores arise at first as erect, dark brown setae. They are subulate, generally straight, 10–14μ wide at the base and 7–8μ wide at the apex. After reaching a length of between 360 and 700μ the apex changes abruptly to a fertile hypha; it is very slightly narrower, paler, generally flexuous or distinctly bent and may reach a length of about 70μ. A branched apex has been observed once only and it was insignificant compared with the branching of the micronematous conidiophores. Conidia are produced on this fertile apex as on the micronematous conidiophores. Some, like setae, remain sterile and end in a very narrow almost hyaline apex. The stromata

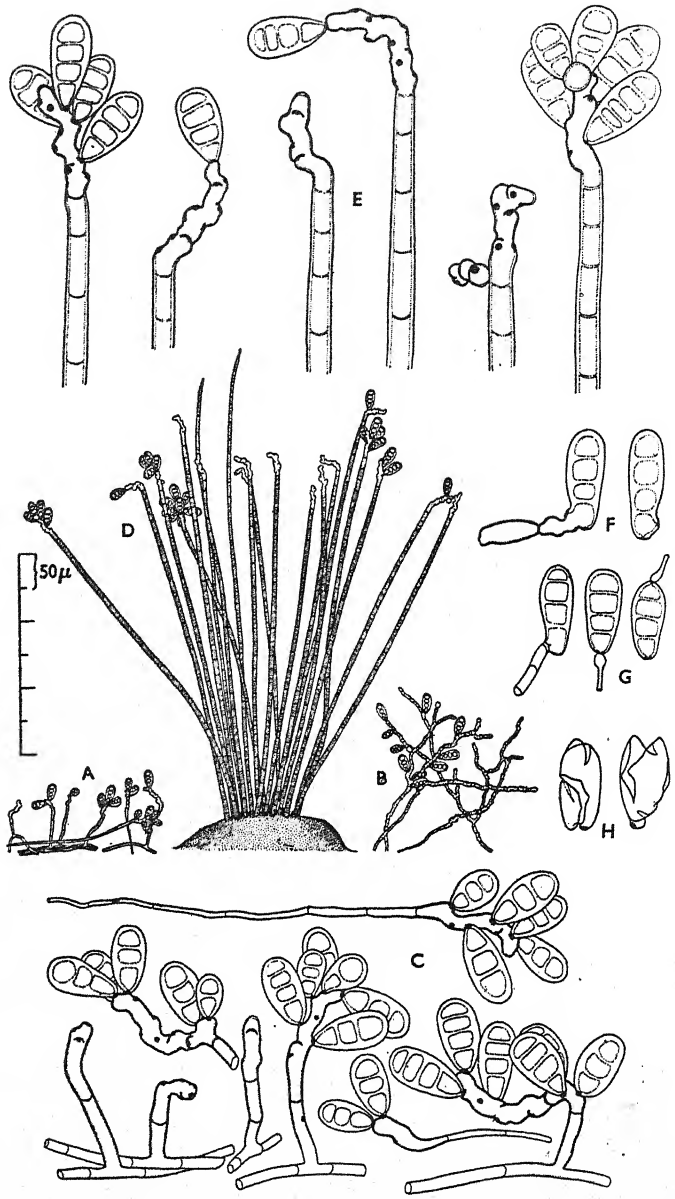


Fig. 5. A, young unbranched micronematous conidiophores, erect or decumbent, ×90; B, older branched micronematous conidiophores, ×90; C, young micronematous conidiophores bearing ears of conidia, ×400; D, erect, divergent macronematous conidiophores on a small stroma, ×90; (A, B and D are drawn to the scale provided on the left); E, fertile apices of macronematous conidiophores and the only 'branched' apex observed is figured second from the right, ×400; F, two unusual conidia, one of which bears two scars, one at the base and the other just above it whilst the second conidium has produced a lateral branch which bears scars, ×400; G, conidia illustrating the three types of germination observed, ×400; H, empty exosporia (or pellicles) of two squashed conidia, ×400. (All figures were drawn from preparations of cultures on Quaker Oat agar.)

grow up from the surface of the agar, become somewhat cylindrical and reach a height of about 500 μ and a width of 200–350 μ.

Fifty conidia from these macronematous conidiophores measured 24–40 by 10–16 μ with a mean of 31 by 13·5 μ; they were 3- to 5-celled, mostly 4-celled, and indistinguishable from those produced on the micronematous conidiophores.

The scars on the conidiophores are as conspicuous as they are on the conidia; they are somewhat raised and dark brown with a paler centre. Conidia do not fall away readily from the conidiophores.

Conidia germinated readily in water (Fig. 5 G) and of a count of 200 germinated conidia 91% showed germ tubes originating through the form exosporium just above the scar; 5·5% showed germ tubes produced from the apical cell; and in 3·5% the germ tubes had emerged through the scar.

Hynes (1937) illustrated a fungus that he had isolated from wheat roots in Australia and which had been identified by Prof. Boedijn as *Curvularia ramosa* (Bainier) Boedijn. This fungus can be passed as a *Curvularia*, as a proportion of the conidia are slightly bent at a somewhat enlarged cell; and it has in fact recently been identified by Groves and Skolko (1945) in Canada as *Curvularia inaequalis* (Shear) Boedijn. However, neither Dr Hynes's fungus nor any other species of *Curvularia* has yet been reported as forming both the micro- and the macro-nematous conidiophores that Bainier described for his *Brachycladium ramosum*. It is, in fact, an infrequently observed character which has, however, been recently recorded for *B. penicillatum* Corda, the type species of *Brachycladium*.

A few possible distinguishing characters between Mr Webster's fungus and '*B. ramosum*' as described by Bainier should perhaps be mentioned. First, according to Bainier, when his fungus was grown on liquorice sticks, the stromata grew up into erect synnemata up to 5 mm. long, over the surface of which unbranched macronematous conidiophores were formed. On the media employed by us, we have not yet seen the stromata of Mr Webster's fungus grow up to a height of 5 mm., but this is not a character to which, at the moment, we attach any significance. Secondly, scars are not indicated in Bainier's figures and it is not clear precisely how the conidia are attached; the bases of some conidia are figured as if tapered and seem to be inserted on to the conidiophores by narrow stalks which can apparently be branched. It is, however, only comparatively recently that a mention of the scar on the conidium or conidiophore has been incorporated into diagnoses, whereas at the present time the nature and number of scars is a character of first importance.

REFERENCES

Bainier, G. (1908). Mycothèque de l'École de Pharmacie—xxiv. *Bull. Soc. mycol. Fr.* xxiv, 73–84.

Boedijn, K. B. (1933). Ueber einige phragmosporen Dematiazeen. *Bull. Jard. Bot. Buitenzorg*, Sér. iii, xiii, Livr. 1, 120–34.

Cooke, M. C. (1871). *Handbook of British Fungi*, ii, p. 564. London.

Groves, J. W. & Skolko, A. J. (1945). Notes on seed-borne fungi. III. *Curvularia*. *Canad. J. Res.* C, xxiii, 94–104.

Hynes, H. J. (1937). Species of *Helminthosporium* and *Curvularia* associated with root-rot of wheat and other graminaceous plants. *J. Proc. Roy. Soc. N.S.W.*, lxx, 378–91.

Smith, A. L. (1903). New or critical microfungi. *J. Bot., Lond.*, xli, 257–60.

THE NUTRITION OF FUNGI FROM THE ASPECT OF GROWTH FACTOR REQUIREMENTS

By NILS FRIES

Uppsala Universitets Institution för fysiologisk Botanik, Uppsala, Sweden

(With 5 Text-figures)

Twelve years ago there would scarcely have been any difficulty in submitting a rather exhaustive paper on the subject 'Growth Factors for Fungi' in the course of three quarters of an hour. It would have been an account of the significance of the so-called bios-substances for the growth of yeast, of the effect of the so-called 'B-growth-substances' on yeast and *Aspergillus niger*, and of a comparatively small number of not very comprehensive studies on similar phenomena in the nutritional physiology of other fungi.

Now, however, it would probably be impossible in the course of an hour even to enumerate the titles of the several hundred articles and books published in this field during the last twelve years; still less would it be possible to record all the results obtained. Moreover, excellent surveys have been published recently, for instance, Schopfer's book *Plants and Vitamins* (1943), and Knight's splendid report on 'Growth Factors in Microbiology' (1945, see also Robbins & Kavanagh, 1942; and Hawker, 1944). Therefore I will confine myself to giving a very summary sketch of the most important results obtained, paying, however, special attention to the results which, at least to my mind, seem to be the most interesting of those made during very recent years.

As early as the eighteen seventies Raulin and other investigators showed that some common moulds, *Aspergillus* and *Penicillium*, could easily be cultivated in simple synthetic nutrient solutions consisting of a sugar and some inorganic salts dissolved in water. These easily cultivated fungi, first and foremost *Aspergillus niger*, came to be the main subject of investigations into the nutrition of fungi for several decades. Thanks to these studies many important problems in the physiology of fungi were elucidated, but it is undeniable that the narrow choice of the species experimented on maintained the conception of sugar being the sole organic compound which fungi required in the substratum.

Early, however, a critical voice made itself heard. Already at the turn of the century the young Belgian scientist Wildiers (1901) considered that his experiments with yeast had indicated the necessity of a certain, chemically undefined substance for the growth of this fungus in a sterile medium. The substance in question, being of organic nature and active in very small quantities, he termed 'bios'. His contemporaries did not agree with his theory, and not until the twenties was the correctness of his conclusions confirmed. At that time one had become accustomed to the concept of

vitamins in animal and human physiology, and the possibility of analogous conditions in microbiology no longer appeared fantastic. The bios active on yeast soon turned out to be divisible into several components termed bios I, bios II, etc., each of which was active separately. In 1928 Miss Eastcott in Canada succeeded in isolating and identifying the factor called bios I, which revealed itself as the well-known compound meso-inositol. Inositol, however, was not absolutely essential for the growth of yeast, but together with other bios-factors not yet isolated, brought about a considerable increase in the rate of growth.

Not until 1934 did the great change occur in our conception of the nutrition of fungi. In that year the Swiss scientist Schopfer proved that thiamin (vitamin B_1), a vitamin of vital importance for man as well as for some animals, was also indispensable to the growth of *Phycomyces*. Next year the Dutch chemist Kögl succeeded in isolating from 40,000 Chinese egg-yolks the perhaps most active bios-factor, bios II, which he called biotin (Kögl, 1935; Kögl & Tönnis, 1936). In a dilution of 1:100,000 million it still proved to be effective and it was obviously indispensable for the growth of yeast, at any rate for the special strain on which he was working.

These sensational discoveries induced numerous mycologists all over the world to test with more exact methods than previously the real demands of different fungi for a synthetic nutrient solution. The results of these studies, carried out during the intervening years, have presented us with quite a new picture of the nutritional physiology of fungi. Different organic compounds, generally called growth factors or growth substances, earlier also bios-substances, nutrilites, etc., have turned out to play the same role for fungi as vitamins play for man and different higher animals, and like them they are active in very small quantities. By far the majority of these growth factors for fungi are identical with different animal vitamins, conspicuously enough, however, only with the water-soluble ones, especially the group of B-vitamins. Although undoubtedly numbers of species within most of the greater groups of fungi have, even after close investigation, proved to be capable of assimilating a simple synthetic solution without any addition of growth factors, in other words are *auxo-autotrophic*, probably even a larger number of species has, nevertheless, turned out to have a demand for one or several such substances, that is, they are *auxo-heterotrophic* (according to Schopfer's terminology).

Table 1. *Growth factors for fungi*

Growth factor	Indispensable for
Thiamin (aneurin, vitamin B_1)	Several phyco-, asco- and basidiomycetes
Biotin (vitamin H)	Several ascomycetes and some basidiomycetes
Pyridoxin (vitamin B_6)	Some ascomycetes
Pantothenic acid	Yeast
Inositol	Some ascomycetes
p-Amino-benzoic acid (vitamin H')	*Rhodotorula aurantiaca*
Hypoxantine (factor ζ_1)	*Phycomyces*: spore germination and early development of mycelium
Oleic acid	*Pityrosporum ovale*
Heteroauxin (β-indolyl-acetic acid)	*Pyronema confluens*? *Tilletia caries*?

Table 1 shows the growth factors of fungi known up till now. On this table the following comments may be made:

Thiamin, the beri-beri-preventing vitamin, is the substance which more often than any other acts as a growth factor. At present over 200 fungi are known requiring thiamin for optimal growth or for growth at all, and it may seriously be questioned whether the number of fungi requiring thiamin is not greater than that capable of growth without this substance. In most cases, as, for instance, *Phycomyces*, only thiamin is necessary, but often other growth factors are needed too.

Biotin, as just mentioned, was originally isolated as a growth factor for yeast, but also functions as a vitamin for higher animals (then often called vitamin H). At least thirty biotin-heterotrophic fungi have been noted hitherto, primarily ascomycetes.

Pyridoxin, vitamin B_6, isolated as late as 1938, was first recognized as a vitamin of vital importance for rats, but is also a growth factor for some fungi, in particular for species of *Ophiostoma*, a few strains of yeast, and dermatomycetes.

Pantothenic acid is as highly effective as biotin and, like it, was isolated as a bios-factor for yeast (by R. J. Williams, 1940). No fungus other than yeast is as yet known upon which it acts as a growth factor, but it is likely that such cases will be found. This growth factor, too, was later established as being a vitamin for different animals.

Inositol acts in considerably larger quantities than the other growth factors just mentioned. It is an indispensable factor for at least two fungi, namely *Nematospora gossypii* (Kögl & Fries, 1937; Fries, 1938) and *Trichophyton discoides* (Robbins, Mackinnon, & Ma, 1942), but strongly stimulates the growth of several others.

p-Amino-benzoic acid, being an important growth factor for some bacteria and a vitamin for higher animals, has, as far as I know, been proved to be a growth factor for only *Rhodotorula aurantiaca* among the fungi (Robbins & Ma, 1944).

Hypoxanthine is a growth factor for *Phycomyces* in the very earliest stage of development, as was shown by Robbins & Kavanagh (1942).

Whether *oleic acid* is to be classed among the true growth factors is questionable. Anyhow, according to Benham (1939, 1941) it is an indispensable factor for the growth of a dermatomycetous fungus, *Pityrosporum ovale*, though in rather large quantities.

Heteroauxin (or β-indolyl-acetic acid) acts like a hormone in higher plants, being effective in very high dilution, but has also been suggested as of importance for the growth of fungi. However, Leonian and Lilly (1937), who tested the activity of heteroauxin on a great number of fungi, obtained consistently negative results. As far as I know, only two experiments indicating a positive effect of heteroauxin on fungi have been published, namely by Kerl (1937) who obtained an increase in the growth rate of *Pyronema confluens*, attaining 176%, and by Defago (1940), who got about six times as much mycelium of *Tilletia caries* (*tritici*) with heteroauxin than without this substance. It is noticeable that in both these cases the substrata were solidified with agar. Repeated experiments seem highly desirable.

The reader will no doubt consider this table quite incomplete. I have, however, quite consciously excluded those growth factors which represent fragments of some of those just discussed—fragments which, in many cases, can be made use of as well as the 'complete', more complicated compound. Thus the two thiamin components, pyrimidin and thiazol, the two pantothenic acid components, β-alanin and pantoic acid, and further pimelic acid, a constituent of biotin, belong to this group. I will account for these interesting substances too in connexion with the biosynthesis of the growth factors.

A fungus, as for instance *Phycomyces*, may be content with only one growth factor for producing optimal or almost optimal growth, or two or several factors may be necessary. Table 2 shows examples of some of the combinations hitherto found of those which are theoretically conceivable as regards the growth substances thiamin, biotin, pyridoxin, and inositol.

Table 2. *Fungi requiring different growth factors or combinations of growth factors*

Growth factors	Fungus	Author reference
B_1	*Phycomyces blakesleeanus*	Schopfer, 1934
B_n	*Melanospora destruens*	Hawker, 1938
B_6	*Ophiostoma catonianum*	Robbins & Ma, 1942*a*
It	—	
B_1+B_n	*Lophodermium pinastri*	Kögl & Fries, 1937
B_1+B_6	*Ophiostoma multiannulatum*	Fries, 1943
B_1+It	—	
B_n+B_6	—	
B_n+It	*Nematospora gossypii*	Kögl & Fries, 1937
B_6+It	—	
$B_1+B_n+B_6$	*Ascoidea rubescens*	Fries, 1943
B_1+B_n+It	—	
B_1+B_6+It	*Trichophyton discoides*	Robbins, Mackinnon & Ma, 1942
B_n+B_6+It	—	
$B_1+B_n+{}_6B+It$	—	

B_1 = thiamin; B_n = biotin; B_6 = pyridoxin; It = inositol

With the aid of the growth factors just enumerated it is probably possible to cultivate most of the known species of fungi under well-defined conditions. It is conspicuous, however, that several fungi grow considerably better, if instead of these growth factors or as an addition, they are furnished with yeast-extract or malt-extract. This effect can of course be explained in different ways: the extract in question may serve as a buffer to the solution, it may contain metals, which are present in the solution in insufficient amounts, and it may contain carbon-, nitrogen-, or sulphur-compounds which are more easily assimilated. There is, however, no doubt that the effect in many cases is brought about by growth factors of a sort as yet unknown.

Such a growth factor is for instance the factor Z_2, which together with hypoxanthine stimulates the germination and the early development of *Phycomyces* according to Robbins's investigations. This factor has not yet been produced in a pure form, but it can be extracted from potatoes and other materials and made into rather concentrated preparations.*

Melin's (1946) investigations into the effect of extracts of leaves on different soil-inhabiting and mycorrhiza-forming fungi also clearly indicate the existence of one or more highly active growth substances in different sorts of litter. This was apparent with particular plainness from experiments with *Clavaria dendroidea*, where the effect of leaf-extracts from different trees was closely analysed. In the extracts at least two different growth factors were found, tentatively called Cl_1 and Cl_2. The latter exerted a pronounced quantitative effect and seemed to be indispensable for the growth of *C. dendroidea*. Certain facts speak in favour of the assumption that the factor Cl_2 may be identical with Robbins's factor Z_2.

Finally, it may be mentioned that the growth-factor requirements of the brewers' yeast are not yet definitively elucidated. The different bios-factors

* In a recent paper Robbins and Bartley Schmidt (1945) suggest that factor Z_2 is identical with a certain combination of amino-acids mainly acting as a buffer.

are certainly identified with inositol, biotin, thiamin, pantothenic acid and pyridoxin, but a great deal of work is still required before one can bring any strain of yeast to optimal growth in a synthetic medium. According to Devloo (1938), one of the remaining factors may be identical with a sterole.

Before I pass on to examine these phenomena from more biochemical and physiological points of view, it may perhaps be profitable to raise the question whether all these results, interesting in themselves, can be of any interest to the systematist or the ecologist.

As to the possible significance of the auxo-heterotrophies for systematics the decisive question is whether each species of fungus is really uniform in regard to its growth-factor requirements. It is generally assumed that this is the case, and that one single strain is thus representative for the species as a whole. In most of the cases more closely examined this assumption has proved to be justified. Thus Blumer (1940) has examined 215 single-cell cultures of the thiamin-heterotrophic *Ustilago violacea* and found all of them identical as to their heterotrophy. The same proved to hold for other species, where two or more different strains were studied. A few exceptions, however, have been noted. Certain strains of *Boletus granulatus* (Melin & Nyman, 1941) have an absolute demand for thiamin, others are able to grow without thiamin, but are strongly stimulated by an addition. The same holds good for different strains of *Marasmius perforans* (Lindeberg, 1944), and concerning pyridoxin for different strains of *Ophiostoma ulmi* (Fries, 1943). According to Robbins & Ma (1941) the demand for biotin of *Fusarium avenaceum* is different in different strains. In all these cases it is not the question of any considerable qualitative but merely quantitative differences in the need of growth factors in different strains.

Thus, as a rule, a distinct type of auxo-heterotrophy seems to distinguish each species, and can be regarded as one of the physiological characteristics of the species, although of course diverging biotypes may originate through mutations and possibly survive for a time.

On the other hand, we have altogether too little evidence from experiment as yet to justify any definite conclusions as to the distribution of the auxo-heterotrophies amongst different groups of fungi. So much we know, however, that quite different types of auxo-heterotrophy are to be found within one and the same genus of fungi. The ascomycetous genus *Ophiostoma*, which has been studied by Robbins and co-workers in New York as well as by us in Uppsala, illustrates this fact. Species representing different combinations and different degrees of thiamin-, biotin-, and pyridoxin-heterotrophy occur in this genus. Another example is of course the genus *Saccharomyces*.

On the whole it appears—if one may judge from the information so far available—as if the most complicated types of auxo-heterotrophies are to be found among the ascomycetes. The phycomycetes are to a large extent auxo-autotrophic or solely thiamin-heterotrophic. The hitherto examined auto-basidiomycetes are all partially or totally thiamin-heterotrophic. Only a few cases of biotin-thiamin-heterotrophy are known within this group. Among the ascomycetes one comes across the most

complicated cases of auxo-heterotrophies like *Ascoidea rubescens* (see Table 2) as well as clearly auxo-autotrophic species, like *Aspergillus niger*.

A priori one would expect the different growth-factor requirements of the fungi to be of interest from an ecological point of view. That does not yet, however, seem to be the case. Thus the hymenomycetes, which are almost without exception thiamin-requiring, represent pronounced saprophytes (e.g., the *Marasmius*-species (Lindeberg, 1944) and most of the Polyporaceae (Fries, 1938)), parasites (such as *Fomes annosus* (Rennerfelt, 1944)), as well as mycorrhiza-forming fungi (such as the *Boleti* (Melin & Norkrans, 1942)). The species of *Ophiostoma* among the ascomycetes, on the other hand, represent quite different types of auxo-heterotrophy, but all of them, except *O. ulmi*, are typical saprophytes. In this connexion, however, one must take into consideration that the growth-factor requirements of the most highly specialized parasitic fungi, the Uredinales as well as Erysiphales, are completely unknown, because nobody has yet has succeeded in bringing them into pure culture. Here perhaps very interesting phenomena remain to be elucidated by future investigators. Then it also seems to me that the cases of complicated auxo-heterotrophy recorded in some dermatomycetes (Mosher, Saunders, Kingery & Williams, 1936; Benham, 1941; Schopfer & Blumer, 1943; and others), might be connected with the manner of living of these skin-specialists.

It was assumed earlier on that the demand for a growth factor in a certain fungus had its cause in a loss of the capacity of synthesizing the factor in question. Thus an auxo-autotrophic species, as for instance *Aspergillus niger*, is a species capable of synthesizing all necessary growth factors by itself. The correctness of this assumption has been confirmed, in perhaps the most elegant way, by so-called 'artificial symbiosis' in which two fungi requiring different growth factors can be cultivated together without any addition of either the one or the other factor because the two symbionts furnish each other with the respective substances (Kögl & Fries, 1937; Schopfer, 1938).

Now it also becomes clear why a growth factor in a certain case can be substituted by another substance the molecule of which represents a fragment of the molecule of the growth factor in question. In such a case the fungus has still intact the capacity of synthesizing a part of the growth-factor molecule. Hence it also shows how the biosynthesis of the growth factors comes to pass by degrees from simple to more and more complex molecules. By observations on the power of different fungi and other organisms to utilize different minor parts of the complete growth-factor molecules, it has consequently been possible to elucidate the pathways for the biosynthesis of the different growth factors.

Concerning the biosynthesis of thiamin we know that the last step in this process consists of a condensation of two, separately formed, simpler substances, namely a pyrimidine- and a thiazole-compound (Fig. 1). This condensation appears to be a reaction rather easily performed by the majority of the fungi investigated, not only the thiamin-autotrophic species, but also most of the thiamin-heterotrophic ones. Thus, an addition of pyrimidine plus thiazole as a rule produces the same effect as a supply of

thiamin. The few exceptional species which are incapable of using the mixture of pyrimidine and thiazole are especially met with in the phycomycetous genus *Phytophthora* (for references see Schopfer, 1943).

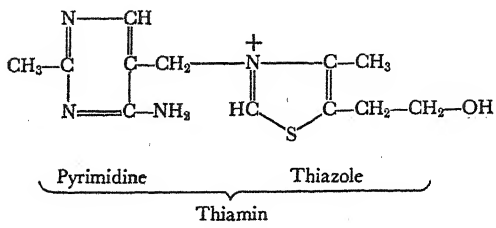


Fig. 1.

Some investigations by Kidder and Dewey (1942) show that a substance named 'Factor *S*', not yet chemically analysed, is necessary for the performance of this last step of the synthesis of thiamin. Some species of *Phytophthora* cannot build up factor *S*, and are therefore not able to link together the two thiamin-components pyrimidine and thiazole into thiamin.

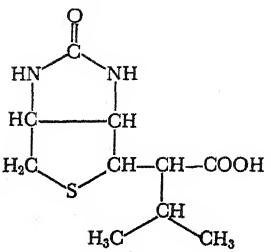
A large number of fungi are capable of synthesizing the thiazole but not the pyrimidine, and at least one fungus, namely *Mucor ramannianus* (Müller & Schopfer, 1937), can synthesize the pyrimidine but not the thiazole. The formation of these two substances is, however, as yet quite uninvestigated. The production of thiazole from thioformamide and aceto-propyl-alcohol which occurs in roots of higher plants has not been observed in fungi (Bonner & Buchman, 1938).

It would carry us too far to take into account the interesting investigations by Bonner and Buchman (1939) as well as by Kavanagh (1942) on the destruction of thiamin which takes place in the fungus cell parallel with its synthesis.

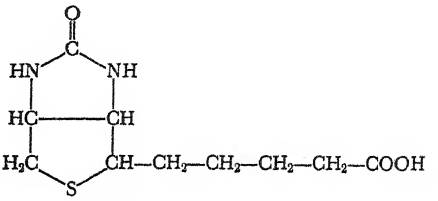
Concerning the biosynthesis of biotin it looks as if pimelic acid should be an important precursor (Fig. 2). Strange to say, an organism exists, namely the diphtheria bacillus, which is capable of synthesizing the complicated heterocyclic part of the biotin-molecule, whereas the long and simple side-chain, the pimelic acid, must be furnished as an external supply (Mueller, 1937). No fungus of a similar type is known: Robbins and Ma (1942*b*) have tested most of the biotin-heterotrophic species of fungi, and in no case could biotin be replaced by pimelic acid as the growth factor. An addition of pimelic acid may, however, stimulate the production of biotin, a fact which is evident from a very interesting experiment performed by R. E. and E. A. Eakin (1942) with *Aspergillus niger*, a totally auxo-autotrophic fungus. They studied the biotin production of this fungus, and found that the production could be increased up to thirty-six times the normal if pimelic acid was added to the medium, an effect clearly indicating the role of a biotin-precursor which pimelic acid plays also within a biotin-autotrophic organism.

We are now able to account for at least some of the details of the biosynthesis of the other, heterocyclic part of the biotin-molecule. Thus it has been found that biotin deprived of the sulphur atom, so-called desthiobiotin, can be utilized by a number of biotin-heterotrophic fungi as well as the complete biotin (Lilly & Leonian, 1944, 1945). Experiments with biotin-heterotrophic mutants, according to methods which I will presently describe, have finally shown, quite definitively, that desthiobiotin is a precursor of biotin at least in certain fungi (Tatum, 1945).

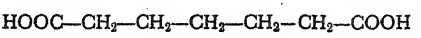
It is perhaps worth while to mention that the biotin originally isolated by Kögl and now called α-biotin diverges from the biotin studied and isolated by American scientists (du Vigneaud, 1942), the so-called β-biotin,



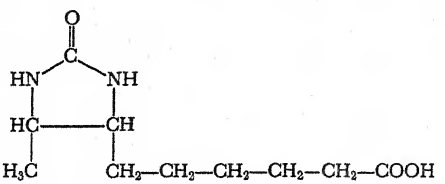
α-Biotin



β-Biotin



Pimelic acid



Desthio-β-biotin

Fig. 2.

in the particular respect that the side-chain—the pimelic acid—is not straight but ramified (Fig. 2). According to Kögl and ten Ham (1943) β-biotin possesses a somewhat higher activity than α-biotin in yeast-growth tests.

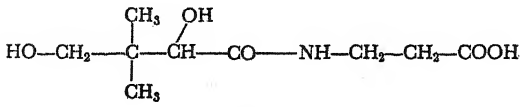
The two precursors of pantothenic acid are β-alanine and α-γ-dihydroxy-ββ-dimethyl-butyric acid—called pantoic acid—which in the fungus cell are condensed to pantothenic acid according to Wieland and Möller (1941) (Fig. 3). As early as 1936 Williams and Rohrman observed the stimulating effect of β-alanine on yeast, and it is obvious that some strains of yeast have a decreased power of synthesizing this substance. No fungus is yet known which—like some strains of the diphtheria bacillus (Mueller & Cohen, 1937)—is entirely incapable of producing β-alanine. The other pantothenic

acid component, pantoic acid, may also function as a growth factor for yeast, at least under special circumstances, according to some results published this year by Hartelius and Johansen (1946).

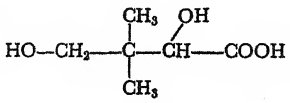
The biosynthesis of pyridoxin (Fig. 4) is still entirely wrapt in obscurity, and the same is true of inositol.

As to *p*-aminobenzoic acid (Fig. 5), certain facts are found which indicate that in the biosynthesis of this substance in the ascomycete *Neurospora* the nitrogen atom is fixed in its final position even before the formation of the ring (Tatum & Beadle, 1942).

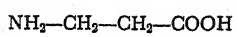
I mentioned that the growth factors are active in very minute quantities. Where a growth substance is absolutely indispensable for the growth of



Pantothenic acid

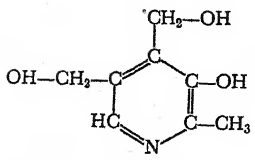


Pantoic acid



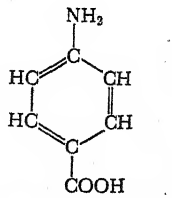
β-Alanine

Fig. 3.



Pyridoxin

Fig. 4.



p-Aminobenzoic acid

Fig. 5.

a fungus it is possible to show, that proportionality exists between the amount of the growth substance added to the culture and the amount of mycelium produced in the culture, or, in other words, the effect of the growth factor is quantitative. I have tried for each growth factor to fix this proportionality in the form of an economic coefficient. This coefficient expresses how much mycelium, dry weight, maximally can be produced, if the growth factor is the only minimum factor, that is, the only factor limiting growth. According to the law of minimum factors (Mitscherlich, 1921) this is valid only—and approximately—when all of the other factors influencing growth are at an optimum. The values obtained give, however, in my opinion, the best quantitative expression of the physiological activity of growth substances (Table 3).

Table 3. *Economic coefficients of different growth factors*

Growth factor	Economic coefficient (average values)	Author reference
Thiamin	400,000–2,500,000	Fries, 1938; Melin & Nyman, 1940; Lindeberg, 1943; Rennerfelt, 1944
Biotin	4,000,000–17,000,000	Lindeberg, 1941, 1946; Fries, 1945*b*
Pyridoxin	300,000–1,400,000	Fries, 1943
Inositol	600–900	Fries, 1938

In this connexion I will draw attention to the fact that the expression 'quantitative effect' here gives the significance valid among Swedish mycologists, namely that the maximal quantities of produced mycelium are proportional to the corresponding quantities of the added growth factor which limits the growth. Some scientists speak of a quantitative effect when the growth rate is proportional to the quantity of the added growth factor. The former interpretation implies an absolute auxo-heterotrophy, the latter not.

Here I should also like to say some words about the term 'growth factor'. As is clear from what I have already said, a growth factor for a fungus is an extremely active substance of organic nature, required by the fungus because the latter has lost the capacity to synthesize it. This definition was originally given by Schopfer, and it is in my opinion the best one. The metals, zinc, molybdenum, etc., similarly active in small quantities, are thus outside this scope, as well as the amino-acids which are active only in greater amounts. The very term 'growth factor' is to my mind, however, not quite good, as those who are not initiated may take it in too wide a sense. A few decades ago the term 'growth factor' was used to symbolize every factor influencing the growth of an organism, thus, for instance, temperature and carbon dioxide tension as well. Hence, in my opinion, the term 'growth substance' would have been preferable, but I think it is too late now to change it.

The question of the most adequate mode of expression is, however, of secondary importance. The growth factors, or growth substances, have turned out to be essential metabolites like, for instance, purins, nucleotides, and amino-acids, and it is scarcely possible to characterize them as a distinctly delimited group. As a summarizing designation for essential metabolites active in small amounts the denomination growth factor might be justified from purely practical reasons, at least as long as the physiological functions of these substances are not finally elucidated.

I will now give a very short account of our present knowledge of the role the growth factors play in the metabolism of fungi.

Thiamin is the growth factor whose physiological functions have been studied most profoundly, and are consequently rather well understood. Investigations with the aid of quite different organisms, primarily yeast fungi, have shown that thiamin is an integral part of the enzyme carboxylase, in the form of thiamin-pyrophosphate, the so-called co-carboxylase (Lohmann & Schuster, 1937). Carboxylase catalyses the de-carboxylation of pyruvic acid, a final stage in the breaking down of sugar. In the absence of thiamin an accumulation of pyruvic acid may occur, as is shown, for

instance, in *Phycomyces* (Haag, 1940). For the function of thiamin in this process the amino-group at C^4 is of crucial importance, and cannot be substituted without a total loss of the physiological activity of the thiamin.

Then thiamin may act in redox-processes in which the thiazole half of the molecule seems to be the more active part (e.g. see Jung, 1940; Karrer, 1944). According to some investigators, the thiazole-ring can be broken up, the nitrogen reduced to its trivalent form, and two molecules of 'thiamin-mercaptane' reversibly joined into a disulphide.

It is likely that thiamin also plays a part in other biochemical processes in the cell.

As to the physiological significance of biotin, an interesting study was published by Winzler, Burk and Du Vigneaud (1944). They closely investigated the effect of biotin on fermentation, respiration, and nitrogen assimilation in a biotin-heterotrophic strain of yeast. They found that if biotin were added to a biotin-free culture of this yeast an increase in the production of carbon dioxide could be recorded even within a few minutes; an increased respiration, on the other hand, was observed only after one hour, and an increased rate of growth as late as after the course of two or three hours. The pre-requisite condition for this rapid rise of the fermentation intensity was, however, that the source of nitrogen should consist of ammonium, for other nitrogen compounds could not make such a rapid effect possible. The authors concluded that it was 'quite possible that biotin in some way brings about the assimilation of ammonia nitrogen which then results in the synthesis of certain materials or enzymes involved in the fermentation system, and thus increases the rate of fermentation, and in turn respiration and growth'.

Concerning the metabolic functions of pantothenic acid one does not know very much as yet. According to Giese and Tatum (1942), who made some experiments with *Neurospora*, pantothenic acid seems to participate in a respiratory system. In the following year Hartelius (1943, 1944) found that an addition of pantothenic acid or β-alanine produced an increased respiration, while on the other hand, the growth stimulation set in only after ten hours. Observations on bacteria give much the same results. So it seems likely that pantothenic acid, like thiamin, is a part of the prosthetic group of some respiratory enzyme.

The role of pyridoxine in the fungus cell is not yet elucidated. Last year, however, several investigations with higher animals or bacteria as experimental objects have shown that pyridoxin participates in the amino-acid metabolism, probably as a co-ferment to one or more transaminases (for references see Wynne, 1946). Thus one may presume that it also fulfils this function in the physiology of fungi.

As regards *p*-amino-benzoic acid and inositol, finally, we know nothing as yet about their physiological activity in the fungus cell.

We have not yet proceeded very far, indeed, in the difficult task of elucidating the roles of the growth factors in the metabolism of fungi. It is evident, however, that the knowledge acquired has mainly been gained thanks to the existence of auxo-heterotrophic organisms. The production of a growth factor essential for a certain biochemical process can usually not

be regulated by man in an auxo-autotrophic organism, and it can be recorded only with difficulty. An auxo-heterotrophic organism, however, is not a producer and a consumer, but merely a consumer as regards one or several growth factors, and so in this case one has the advantage of counting the substance, or substances, in question among the external factors, or environmental factors, which are entirely controllable.

The material offered by auxo-heterotrophic organisms is really surprisingly rich, and its mapping is as yet certainly far from being complete, but it seems to be a question of losses in the synthesizing power concerning a relatively small number of growth factors and similar substances. There is every reason for the assumption that an immensely greater number of substances of equal importance are synthesized and function in the living cells, although these substances never adopt the character of growth factors. Why they do not, can of course be explained in many different ways, but one possibility is that the loss of the synthetic power as to certain essential metabolites, brings about the rapid death of the organism because of the impossibility of procuring these substances from the substratum.

It is, however, quite clear that such new strains requiring other growth factors than those hitherto known, if they could be kept alive, would present extraordinarily valuable material for science. This idea might perhaps have been the starting-point for the very interesting and significant experiments which were initiated in 1941 by the American scientists: Beadle, a geneticist, and Tatum, a biochemist; and which to my mind inaugurate a new epoch in the investigating of the nutrition of fungi. By X-ray or ultra-violet irradiation of young perithecia or microconidia of *Neurospora crassa* they produced mutations in this fungus manifesting themselves as new auxo-heterotrophies (beyond the biotin-heterotrophy which normally characterizes *Neurospora*). The first mutations of this kind—so-called biochemical or physiological mutations—which they obtained, concerned pyridoxin, *p*-amino-benzoic acid, and the thiazole-component of thiamin (Beadle & Tatum, 1941). Later on they also obtained thiamin-, pantothenic-, niacin-, lactoflavin-, and choline-heterotrophic mutants, the three last-mentioned cases thus referring to substances not found in nature as growth factors for fungi. All these mutants could be kept alive without difficulty, if they were cultivated on a substratum containing the growth factor in question. In addition to these auxo-heterotrophic strains they isolated several mutants which had lost the ability of synthesizing one or another amino-acid, a nucleotide constituent, etc. I shall not enter deeply into these *Neurospora*-studies, certainly well known to you (for references see Horowitz, Bonner, Mitchell, Tatum & Beadle, 1945; Beadle & Tatum, 1945). I will confine myself to emphasizing the new possibilities now opened for studying the biosynthesis of the growth factors and their physiological functions in the organism. As I have already mentioned, any such biosynthesis can be viewed as a consecutive series, or several converging series of biochemical reactions. As is clear from what we already know, for instance about the thiamin-synthesis, a biosynthesis can be blocked in one or more particular reactions, often not the same in different

organisms, and through comparative studies the different steps of the biosynthesis can be elucidated. 'Instead of comparing different organisms which may vary in several respects, there are', as Tatum (1944) has pointed out, 'a number of advantages in comparing different strains of the same organism, especially if these vary in only a single reaction. . . .' With the aid of mutants of *Neurospora* one has thus succeeded in making clear the synthesis of the amino-acids arginine and tryptophane in the fungus cell, and also in elucidating the biosynthesis of different growth factors in certain points. The latter results I have already briefly mentioned.

It appears, however, also from *Neurospora*, how difficult, if not impossible, it is to mark out any sharp boundary line between growth factors and other organic substances necessary for the organism. Pantothenic acid and pyridoxin are as a matter of course to be looked upon as growth substances of certain mutant strains of *Neurospora*, but is that true regarding cytidylic acid, adenine, or choline as well, which are effective in somewhat greater amounts? Here we have a rather continuous series of 'economic coefficients', and it must be a matter of judgement where the dividing line shall be fixed between growth factors and substances active in greater amounts. The main point is, as is emphasized in particular by Knight (1945), that all these organic compounds are essential metabolites.

It is quite evident that the more mutants one can isolate within each special type of auxo-heterotrophy, the greater are the chances of getting representatives of different blockings in the chain of biosynthesis. The mutants of *Neurospora* produced by the irradiation methods hitherto employed are deficient primarily in amino-acids, in the next place in real growth factors, and then there are a few mutants deficient as regards nucleotides and other metabolites as yet partly unknown. Only a few organisms other than *Neurospora* have been tested so far, and in most of these the material is still too limited to justify any comparisons. Thus some physiological mutations have been produced in *Penicillium chrysogenum* by Beadle and co-workers (unpublished, cited from Tatum, 1945), in *Aspergillus terreus* by Hollaender and co-workers (Hollaender, Raper & Coghill, 1945), and in *Absidia* by Tatum's group at Yale.

During recent years I have isolated and examined a series of physiological mutants of the ascomycete *Ophiostoma multiannulatum*, a normally thiamin-pyridoxin-heterotrophic fungus (Fries, 1945*a*, *b*; 1946*a*, *b*). I will conclude my lecture by showing a table summarizing the experiments I have made for the purpose of isolating greater amounts of physiological mutants according to a method of concentrating the mutants, or another rather handy method of total isolation. By the aid of these methods, unnecessary to describe here, I have—mainly during the last three months—isolated over 500 mutants, 408 of which now are at least tentatively classified. It may perhaps be of interest to compare the qualitative composition of the mutant yields in the two cases *Ophiostoma* and *Neurospora*. Unfortunately, there is no general survey published of all mutants of *Neurospora* isolated and identified, but I have tried to produce a collection by the help of different articles published on this subject (Table 4).

A direct comparison is justified only between the 'totally isolated'

mutants of *Ophiostoma* and those of *Neurospora*, since an elimination of certain mutants is inevitable in the experiments by the concentration method. The total sums are, however, included in one of the columns.

The table shows that the amino-acid deficiencies are predominant in both fungi, but particularly it seems in *Neurospora*. Especially characteristic of *Ophiostoma* are the so-called *p*-thiotrophic mutants which are incapable

Table 4. *A tabular summary of the physiological mutants obtained in* Ophiostoma *and* Neurospora

	Number of mutants isolated			
	Ophiostoma			
	Isolation method			
Essential substance	'Concentration'	'Total isolation'	Sum total	*Neurospora*
Reduced nitrogen	—	—	—	2
Reduced sulphur	40	26	66	—
An amino-acid:				
Arginine	56	35	91	>30
Lysine	22	20	42	40
Leucine	—	—	—	+
Methionine	8	9	17	>50
Isoleucine+valine	—	—	—	1
Cystine	—	—	—	+
Tryptophane	—	—	—	33
Proline	—	—	—	+
Threonine	—	—	—	+?
Serine	—	—	—	+?
Phenylalanine	—	—	—	+?
Asparagine	—	1	1	—
Not identified	3	1	4	—
A nucleotide constituent:				
Uracil	15	17	32	3
Hypoxanthine	47	42	89	2
Adenine	3	3	6	—
Guanine	4	—	4	—
A vitamin (growth factor):				
Biotin	3	2	5	*
Thiamin	*	*	*	6
Pyridoxin	*	*	*	+
p-Amino-benzoic acid	4	—	4	+
Pantothenic acid	—	6	6	2
Inositol	2	17	19	5
Nicotinic acid	2	3	5	3
Lactoflavin	—	—	—	1
Choline	—	—	—	5
Unknown factor	9	8	17	+
Sum total	218	190	408	>380

* A deficiency characteristic of the species

of reducing six-valent sulphur. Then, losses of the synthetic power regarding different constituents of nucleotides—particularly hypoxanthine—are considerably more common in *Ophiostoma*. In both cases the induced auxoheterotrophies represent a minority—though an interesting minority. The group named 'Unknown factor' will, however, perhaps turn out to comprise the most interesting types of mutants.

As appears from the table, *Ophiostoma* partly furnishes other types of physiological mutations than *Neurospora*. The purin- and pyrimidine-deficient strains seem to be of special interest, and so they are at present being investigated in our laboratory in Uppsala. One can assume that other fungi may produce mutations interesting in other respects.

The exceptional possibilities which fungi offer to studies of induced physiological mutations, and the possibilities such mutations offer in elucidating metabolic processes makes it likely that our knowledge of the physiology of fungi will be widened before long to an unimagined degree. The progress in this domain will then no doubt be of advantage to other branches of science. It will be possible to work out new valuable chemotherapeutica according to the principle of anti-vitamins. Biochemists and food-chemists will obtain specific and rapidly registrating microbiological test-objects for different amino-acids, vitamins, etc. Above all, however, we hope to break still more of the numerous seals hiding the big secret, the problem of life.

REFERENCES

BEADLE, G. W. & TATUM, E. L. (1941). Genetic control of biochemical reactions in *Neurospora*. *Proc. Nat. Acad. Sci.* XXVII, 499.

BEADLE, G. W. & TATUM, E. L. (1945). *Neurospora*. II. Methods of producing and detecting mutations concerned with nutritional requirements. *Amer. J. Bot.* XXXII, 678.

BENHAM, R. W. (1939). The cultural characteristics of *Pityrosporum ovale*—a lipophylic fungus. *J. Investigative Dermatology*, II, 187.

BENHAM, R. W. (1941). Cultural characteristics of *Pityrosporum ovale*—a lipophylic fungus. Nutrient and growth requirements. *Proc. Soc. exp. Biol. Med.* XLVI, 176.

BLUMER, S. (1940). Über die Aneurinheterotrophie bei einigen antheren-bewohnenden Brandpilzen. *Mitt. Naturforsch. Ges. Bern*, XIX.

BONNER, J. & BUCHMAN, E. R. (1938). Synthesis carried out in vivo by isolated pea roots. I. *Proc. Nat. Acad. Sci. U.S.A.* XXIV, 431.

BONNER, J. & BUCHMAN, E. R. (1939). The synthesis and destruction of vitamin B_1 by *Phycomyces*. *Proc. Nat. Acad. Sci. U.S.A.* XXV, 164.

DÉFAGO, G. (1940). Effets de l'aneurine, de ses composants et de l'hétéro-auxine sur la croissance de trois parasites du blé. *Phytopath. Z.*, XIII, 293.

DEVLOO, R. (1938). Un stérol indispensable à la levure. *Arch. internat. Physiol.* XLVI, 157.

DU VIGNEAUD, V. (1942). The structure of biotin. *Science*, XCVI, 455.

EAKIN, R. E. & EAKIN, E. A. (1942). A biosynthesis of biotin. *Science*, XCVI, 187.

EASTCOTT, E. V. (1928). Wildiers' bios. The isolation and identification of 'bios I'. *J. physical Chemistry*, XXXII, 1094.

FRIES, N. (1938). Über die Bedeutung von Wuchsstoffen für das Wachstum verschiedener Pilze. *Symb. Bot. Upsal.*, III, no. 2.

FRIES, N. (1943). Die Einwirkung von Adermin, Aneurin und Biotin auf das Wachstum einiger Ascomyceten. *Symb. Bot. Upsal.* VII, no. 2.

FRIES, N. (1945*a*). X-ray induced mutations in the physiology of *Ophiostoma*. *Nature*, CLV, 757.

FRIES, N. (1945*b*). Two X-ray induced auxo-heterotrophies. *Svensk Bot. Tidskr.* XXXIX, 270.

FRIES, N. (1946*a*). X-ray induced parathiotrophy in *Ophiostoma*. *Svensk Bot. Tidskr.* XL, 127.

FRIES, N. (1946*b*). Mutant strains of *Ophiostoma multiannulatum* requiring components of different nucleotides. *Ark. f. Bot.* XXXIII A, nr. 7.

GIESE, A. C. & TATUM, E. L. (1942). The effect of some vitamins of the B-complex on respiration of mutants of *Neurospora*. *Collecting Net.* XVII, 86.

HAAG, E. (1940). Sur le mécanisme du test *Phycomyces* de l'aneurine. *C.R. Soc. Phys. Hist. Nat. Genève*, LVII, 136.

HARTELIUS, V. (1943). Einfluss des β-Alanins auf Atmung und Gärung der Hefe bei kurzdauernden Versuchen. *Naturwiss*, XXXI, 139.

HARTELIUS, V. (1944). Beschleunigung der Wirkung von β-Alanin auf die Atmung und das Wachstum der Hefe durch Dioxy-dimethyl-buttersäure. *Naturwiss.* XXXII, 81.

HARTELIUS, V. & JOHANSEN, G. (1946). The influence of α, γ-dihydroxy-β, β-dimethyl-butyric acid on yeast growth. *C.R. Lab. Carlsberg*, XXIV, nr. 12.

HAWKER, L. E. (1938). Effect of growth substances on growth and fruiting of *Melanospora destruens*. *Nature*, CXLII, 1038.

HAWKER, L. E. (1944). Fungi and vitamins. *J. Roy. Coll. Sci.*, XIV, 65.

HOLLAENDER, A., RAPER, K. B. & COGHILL, R. D. (1945). Production and characterization of ultraviolet-induced mutations in *Aspergillus terreus*. I. Production of mutations. *Amer. J. Bot.* XXXII, 160.

HOROWITZ, N. H., BONNER, D., MITCHELL, H. K., TATUM, E. L. & BEADLE, G. W. (1945). Genetic control of biochemical reactions in *Neurospora*. *Amer. Nat.* LXXIX, 304.

JUNG, A. (1940). Die Funktion der Vitamine des B-Komplexes im Organismus. *Z. f. Vitaminforsch., Suppl. I*, 1.

KARRER, P. (1944). Kurze Ausschnitte aus der Chemie und Biochemie der Vitamine: Nicotinsäureamid, B_1, B_2, B_6, Pantothensäure und Biotin. *Schweiz. Z. Pathol. u. Bakt.* VII, 303.

KAVANAGH, F. (1942). The interaction between thiamine and four fungi. *Bull. Torrey bot. Club*. LXIX, 669.

KERL, I. (1937). Über Regenerationsversuche an Fruchtkörpern und andere entwicklungsphysiologische Untersuchungen bei *Pyronema confluens*. *Z. f. Bot.* XXXI, 129.

KIDDER, G. W. & DEWEY, V. C. (1942). The biosynthesis of thiamin by normally athiaminogenic micro-organisms. *Growth*, VI, 405.

KNIGHT, B. C. J. G. (1945). Growth factors in microbiology. Some wider aspects of nutritional studies with micro-organisms. *Vitamins and hormones*, III, 105.

KÖGL, F. (1935). Wuchsstoffe der Auxin- und der Bios-Gruppe. *Ber. dtsch. bot. Ges.* LXVIII A, 16.

KÖGL, F. & FRIES, N. (1937). Über den Einfluss von Biotin, Aneurin und Meso-Inosit auf das Wachstum verschiedener Pilzarten. *Z. physiol. Chemie*, CCXLIX, 93.

KÖGL, F. & TEN HAM, E. J. (1943). Über die Verschiedenheit der aus Eigelb und Leber isolierten Biotin-Kristallisate. *Naturwiss.* XXXI, 208.

KÖGL, F. & TÖNNIS, B. (1936). Über das Bios-Problem. Darstellung von krystallisiertem Biotin aus Eigelb. *Z. physiol. Chemie*, CCXLII, 43.

LEONIAN, L. H. & LILLY, V. G. (1937). Is heteroauxin a growth-promoting substance? *Amer. J. Bot.* XXIV, 135.

LILLY, V. G. & LEONIAN, L. H. (1944). The anti-biotin effect of desthiobiotin. *Science*, XCIX, 205.

LILLY, V. G. & LEONIAN, L. H. (1945). A quantitative comparison of biotin and desthiobiotin for the growth of certain micro-organisms. *Proc. West Va. Acad. Sci.* XVII, 55. (in *Bull. W. Va. Univ.* Ser. 45, 1945).

LINDEBERG, G. (1941). Über die Wirkung von Biotin auf *Marasmius androsaceus* (L.) Fr. *Arch. Mikrobiol.* XII, 58.

LINDEBERG, G. (1944). Über die Physiologie ligninabbauender Boden-Hymenomyzeten. Studien an schwedischen *Marasmius*-Arten. *Symb. Bot. Upsal.* VIII, no. 2.

LINDEBERG, G. (1946). The effect of biotin and thiamin on the growth of *Collybia dryophila* Fr. *Svensk Bot. Tidskr.* XL, 63.

LOHMANN, K. & SCHUSTER, PH. (1937). Über die Co-Carboxylase, *Naturwiss.* XXV, 26.

MELIN, E. (1946). Der Einfluss von Waldstreuextrakten auf das Wachstum von Bodenpilzen, mit besonderer Berücksichtigung der Wurzelpilze von Bäumen. *Symb. Bot. Upsal.* VIII, no. 3.

MELIN, E. & NORKRANS, B. (1942). Über den Einfluss der Pyrimidin- und der Thiazolkomponente des Aneurins auf das Wachstum von Wurzelpilzen. *Svensk Bot. Tidskr.* XXXVI, 271.

MELIN, E. & NYMAN, B. (1940). Weitere Untersuchungen über die Wirkung von Aneurin und Biotin auf das Wachstum von Wurzelpilzen. *Arch. Mikrobiol.* XI, 318.

MELIN, E. & NYMAN, B. (1941). Über das Wuchsstoffbedürfnis von *Boletus granulatus* (L.) Fr. *Arch. Mikrobiol.* XII, 254.
MITSCHERLICH, E. A. (1921). Das Wirkungsgesetz der Wachstumsfaktoren. *Landw. Jahrb.* LVI, 71.
MOSHER, W., SAUNDERS, D., KINGERY, L. & WILLIAMS, R. (1936). Nutritional requirements of the pathogenic mold *Trichophyton interdigitale*. *Plant Physiol.* XI, 795.
MUELLER, J. H. (1937). Studies on cultural requirements of bacteria. X. Pimelic acid as a growth stimulant for *C. diphtheriae*. *J. Bact.* XXXIV, 163.
MUELLER, J. H. & COHEN, S. (1937). Beta alanine as a growth accessory for the diphtheria bacillus. *J. Bact.* XXXIV, 381.
MÜLLER, W. & SCHOPFER, W. H. (1937). L'action de l'aneurine et de ses constituants sur *Mucor Ramannianus* Möll. *C.R. Acad. Sci., Paris*, CCV, 687.
RENNERFELT, E. (1944). Die Entwicklung von *Fomes annosus* Fr. bei Zusatz von Aneurin und verschiedenen Extrakten. *Svensk Bot. Tidskr.* XXXVIII, 153.
ROBBINS, W. J. & BARTLEY SCHMIDT, M. (1945). Factor Z_2 and gametic reproduction by *Phycomyces*. *Amer. J. Bot.* XXXII, 320.
ROBBINS, W. J. & KAVANAGH, F. (1942). Hypoxanthine, growth substance for *Phycomyces*. *Proc. Nat. Acad. Sci., U.S.A.* XXVIII, 65.
ROBBINS, W. J. & KAVANAGH, V. (1942). Vitamin deficiencies of the filamentous fungi. *Bot. Rev.* VIII, 411.
ROBBINS, W. J. & MA, R. (1941). Biotin and growth of *Fusarium avenaceum*. *Bull. Torrey bot. Club*, LXVIII, 446.
ROBBINS, W. J. & MA, R. (1942*a*). Vitamin deficiencies of *Ceratostomella* and related fungi. *Amer. J. Bot.* XXIX, 835.
ROBBINS, W. J. & MA, R. (1942*b*). Pimelic acid, biotin and certain fungi. *Science*, XCVI, 406.
ROBBINS, W. J. & MA, R. (1944). A *Rhodotorula* deficient for para-amino-benzoic acid. *Science*, C, 85.
ROBBINS, W. J., MACKINNON, J. E. & MA, R. (1942). Vitamin deficiencies of *Trichophyton discoides*. *Bull. Torrey bot. Club*, LXIX, 509.
SCHOPFER, W. H. (1934). Versuche über die Wirkung von reinen kristallisierten Vitaminen B auf *Phycomyces*. *Ber. dtsch. bot. Ges.*, LII, 308.
SCHOPFER, W. H. (1938). Symbiose et facteurs de croissance. *C.R. 1er Congrès des microbiologistes de langue française*, Paris.
SCHOPFER, W. H. (1943). *Plants and vitamins*. Waltham, Mass., U.S.A.
SCHOPFER, W. H. & BLUMER, S. (1943). Zur Wirkstoffphysiologie von *Trichophyton album* Sab. *Ber. Schweiz. Bot. Ges.* LIII, 409.
TATUM, E. L. (1944). Biochemistry of fungi. *Ann. Rev. Biochem.* XIII, 667.
TATUM, E. L. (1945). Desthiobiotin in the synthesis of biotin. *J. Biol. Chem.* CLX, 455.
TATUM, E. L. & BEADLE, G. W. (1942). Genetic control of biochemical reactions in *Neurospora*: an 'amino-benzoicless' mutant. *Proc. Nat. Acad. Sci., U.S.A.* XXVIII, 234.
WIELAND, T. & MÖLLER, E. F. (1941). Über eine biologische Synthese der Pantothensäure. *Z. physiol. Chemie*, CCLXIX, 227.
WILDIERS, E. (1901). Nouvelle substance indispensable au développement de la levure. *La Cellule*, XVIII, 311.
WILLIAMS, R. J. (1940). Pantothenic acid. *Enzymologia*, IX, 387.
WILLIAMS, R. J. & ROHRMAN, E. (1936). Beta-alanine and bios. *J. Amer. Chem. Soc.* LVIII, 695.
WINZLER, R. J., BURK, D. & DU VIGNEAUD, V. (1944). Biotin in fermentation, respiration, growth and nitrogen assimilation by yeast. *Arch. Biochem.* V, 25.
WYNNE, A. M. (1946). Non-oxidative enzymes. *Ann. Rev. Biochem.* XV, 35.

THE EFFECT OF CERTAIN GROWTH SUBSTANCES ON MYCELIAL GROWTH AND FRUITING OF *MELANOSPORA DESTRUENS* SHEAR

By LILIAN E. HAWKER
Department of Botany, University of Bristol

(With Plates VI and VII)

The effects of interaction between two organisms growing on the same culture plate have often been noted by mycologists. At present interest is focused on the antagonistic effect of one organism upon another, and it need not be emphasized that the investigation of antagonism between micro-organisms has yielded results of great practical importance. The stimulation of one organism by another is equally frequent and is not without practical significance. There are numerous references in mycological literature to the greater abundance of mycelium or spores of certain fungi in the presence of a second micro-organism.

When newly isolated, a strain of *Melanospora destruens* Shear from apple fruit produced its perithecia freely on malt extract agar, less freely on potato extract agar and only sparsely on certain synthetic (glucose, salts) media* (Pl. VI, fig. 1). Plates of the latter which had become contaminated, after being opened for examination, showed a distinct ring of perithecia surrounding the intruder colonies and contrasting with the rest of the plate where fruit bodies were few and scattered. The effects of a number of fungi grown in mixed culture with *M. destruens* were examined (Asthana & Hawker, 1936). Large Petri dishes were poured with a glucose-salts medium (medium A = glucose, 5 g.; KNO_3, 3·5 g.; KH_2PO_4, 1·75 g.; $MgSO_4$, 0·75 g.; agar powder, 15 g.; distilled water, 1 litre), and were inoculated with *M. destruens* and one of a number of other fungi. The majority of the fungi examined definitely stimulated the production of perithecia by *M. destruens*. Some caused the production of a distinct ring of perithecia round their margins (e.g. *Penicillium* sp. and *Monilia fructigena*, Pl. VI, figs. 2, 3). With others there was a clear zone of medium between the 'intruder' colony and that of *Melanospora* across which neither mycelium could pass. Perithecia of *M. destruens* were formed where its mycelium reached the clear zone (e.g. *Sclerotinia trifoliorum*, Pl. VI, fig. 4). The hyphae of others intermingled with those of *Melanospora* and the perithecia of the latter were formed over the area occupied by the intruder (e.g. *Sphaeropsis malorum*, Pl. VI, fig. 5). Certain fungi which grew slowly on medium A were unable to become established before the whole of the plate was occupied by the fast-growing *Melanospora*. Some of these showed a stimulatory effect if they were planted a few days before the latter (e.g. *Pesta-*

* During the investigation the fungus gradually lost the ability to fruit and finally to grow on a synthetic medium without the addition of growth substances.

lozzia hartigii). Conversely, certain fungi with very rapid growth prevented the development of the *Melanospora* if planted on the same day, but stimulated perithecial production if planted one to two days later (e.g. *Mucor hiemalis*). Yet others were unable to grow on medium A. Some of these were stimulatory on other media such as dilute malt extract agar.

Fungi which showed a particularly stimulatory effect on perithecial production were grown on medium A without agar. Seven to ten days after inoculation the medium was filtered and autoclaved after the addition of agar. A segment was removed from a plate of medium A previously inoculated with *Melanospora destruens*, and was replaced by a segment of the used medium of similar size and shape. Fruiting was stimulated when the fungus grew on to this segment of used medium. Whole plates of the used medium were also inoculated with *M. destruens*. Perithecia were produced earlier and in greater number on these than on medium A unless the used medium had been prepared from old cultures in which, presumably, toxic staling substances had accumulated.

Thus the presence of the living fungus was not essential to produce the stimulatory effect, but the stimulating organisms had modified the medium in such a way that it became more suitable for the fruiting of *M. destruens*. This modification might have been due to a reduction in the concentration of nutrients in the medium, or to the synthesis by the first fungus of some substance or substances which stimulated fruiting, but which were not readily formed by *M. destruens*, in pure culture. Further experiments were designed to test these possibilities. The production of perithecia was increased by diluting medium A to between one-third and two-thirds of its original concentration or by a similar reduction in the glucose content (Pl. VI, fig. 6). Removal of glucose from the medium by the first fungus could not entirely account for the degree of stimulation. Moreover, the stimulatory effect increased or decreased with concentration or dilution of the used medium. The addition of a small amount of glucose to the latter increased fruiting but further increases reduced it. The used media were fractionated with ether at pH 5–6 and the two fractions were freed from ether and solidified with agar. The ether-insoluble fraction stimulated fruiting to a much greater extent than did either the original used medium or one in which the two fractions were recombined (Pl. VII, fig. 7). The ether-soluble fraction either prevented fruiting (when it had been prepared from a relatively old culture) or gave the same effect as a dilute medium (Pl. VII, fig. 8), because the residual nutrients from the used medium remained in the ether-insoluble fraction. Under the conditions of the fractionation certain staling products, such as organic acids produced by the first fungus, would presumably have been extracted by the ether, and this would account both for the superiority of the ether-insoluble fraction compared with the used medium itself, and for the inhibitory effect of the ether-soluble fraction of 'stale' culture media.

These experiments show that the used media influenced fruiting in three ways: (*a*) by the presence of a definite stimulatory substance or substances synthesized by the first fungus, (*b*) by the reduced concentration of the original nutrients, particularly of glucose, in the medium, and (*c*) by the

presence of inhibitory metabolic substances. The intensity of fruiting on a used medium was thus due to a combination of these three factors. A used medium from a very young culture had little effect on perithecial production, for neither the stimulatory nor the inhibitory substances had accumulated to any extent. In a slightly older culture the stimulatory substances had presumably accumulated to a significant extent, while toxic metabolic products had not yet reached an inhibiting concentration. Moreover, sufficient glucose and other nutrients had been removed to give a further increase in fruiting. Later the used medium inhibited fruiting and, later still, growth of *M. destruens*, owing to the complete removal of food and to the high concentration of toxic staling products.

Meanwhile, Buston and his co-workers (1931*a*, *b*, 1933) had been investigating the accessory food factors required by *Nematospora gossypii* and had shown that an excellent source of these was an extract of lentils. This extract was tested on *Melanospora destruens* with striking results. The perithecial frequency (determined by a method of counting perithecia in arbitrarily fixed microscopic fields) in a nine-day-old culture was increased from 0 on medium A to 10·2 on the same medium with the addition of 0·2% dry weight of lentil extract (Hawker, 1936). Pl. VII, fig. 9 shows the effect of replacing a segment of medium A by one of the lentil medium. Buston had shown that the crude lentil extract could be fractionated with barium hydroxide to give two fractions, both of which were essential for growth of *Nematospora gossypii*. The precipitate fraction was identified as *i*-inositol. These fractions were tested on *Melanospora destruens*, and it was shown that inositol had no visible effect while the inositol-free fraction was as effective as the original extract or as the two fractions recombined.

The addition of glucose to medium A plus lentil extract had a depressing effect on fruiting similar to that of adding glucose to a used medium. With either lentil extract or used medium as a source of the stimulatory substance, an increase in the amount of the latter raised the concentration of glucose optimal for fruiting. Comparable results were obtained with certain other fungi and bacteria. The similarity in behaviour of the lentil extract and the used media led to the conclusion that the same active factor was present.

Later the brilliant work of Kögl and Fries (1937) showed that with *Nematospora gossypii* the active factor in the barium hydroxide filtrate fraction was biotin, which had previously been isolated from egg-yolk by Kögl and Tönnis (1936). (Biotin has since been identified with vitamin H by György *et al.* (1940).) This fraction also contained aneurin (thiamin, vitamin B_1). Growth of *N. gossypii* was good in the presence of inositol and biotin and was further increased by the addition of aneurin. Kögl and Fries showed by an ingenious experiment with mixed cultures of *N. gossypii* and *Polyporus adustus*, which is known to require an external supply of aneurin, that the former was able to synthesise a suboptimal amount of this vitamin.

A small quantity of pure biotin methyl ester was kindly supplied by Prof. Kögl. *Melanospora destruens* grew fairly well on medium A plus biotin but did not fruit (Pl. VII, fig. 10). (By this time it produced only negligible growth and no fruit bodies on medium A alone.) The addition of aneurin

to medium A gave no result, but the addition of both aneurin and biotin to the medium caused good mycelial growth and the production of numerous perithecia (Pl. VII, figs. 11, 12). The addition of inositol to medium A plus biotin plus aneurin gave no further increase in growth or fruiting. The thiazole component of aneurin was inactive but the pyrimidine component gave a result comparable to that obtained with aneurin or with a mixture of the two components (Table 1).

Table 1.

Medium	Dry wt. mycelium* (mg. per 100 c.c. medium after 1 week)	Perithecial frequency after 1 week
A	Negligible	None
A + inositol (20 mg. per 100 c.c. medium)	Negligible	None
A + biotin (4γ per 100 c.c. medium)	24	None
A + aneurin (4γ per 100 c.c. medium)	Negligible	None
A + biotin + aneurin	165	4·6
A + biotin + aneurin + inositol	162	4·8
A + biotin + thiazole	—	None
A + biotn + pyrimidine	—	4·3
A + biotin + thiazole + pyrimidine	—	4·7
A + inositol-free fraction of lentil extract	142	1·9

* Dry wt. of mycelium was obtained from liquid cultures, perithecial frequency from parallel agar cultures.

By means of mixed culture experiments with *M. destruens* and *Nematospora gossypii* it was shown that the former is able to synthesize inositol.

It has already been stated that the amount of glucose optimal for fruiting increased with increase in concentration of lentil extract or of a medium previously used by another fungus. The optimal amount of glucose was similarly increased by increase in the concentration of aneurin both with *Melanospora destruens* (Hawker, 1939) and with certain other fungi (Hawker, 1942). Concentration of biotin had no such effect. These results suggested that either the rate of utilization of glucose increased with the addition of aneurin or that the vitamin acted on the fungus in some way, tending to make it more tolerant of high concentration of glucose. The rate of removal of glucose from the medium was examined by direct estimation of the residual sugar (Hawker, 1944). The ratio of glucose used to dry weight of mycelium increased considerably with increase in aneurin concentration. Intake of oxygen was then measured by a manometric method and a significant increase in the rate of respiration in the presence of aneurin was demonstrated. Since the substrate was glucose it was assumed that the ratio of oxygen absorbed to carbon dioxide given off would be unity, and the results were expressed as milligrams of carbon dioxide per gram dry weight of mycelium per hour. The mean of ten experiments gave an increase in this ratio of 6·74–12·65 with an increase in amount of aneurin from 1 to 11 γ/100 c.c. medium, which was shown to be significant.

A further investigation of the possible correlation between respiration rate and fruiting of certain fungi is in progress. Unpublished data show that a high rate of respiration on a sucrose medium compared with a glucose

medium of the same concentration is correlated with a poorer development of mycelium and a greater number of perithecia on the former.

In this connexion it is of interest that at a recent informal discussion on the ecology of the higher fungi during the Society's Autumn Foray at Whitby in 1946 it was suggested that the scarcity of agarics after such an exceptionally wet summer was due to the low rate of respiration of the subterranean mycelium in a saturated soil.

A detailed investigation of the contrasting effects of glucose and sucrose on growth and fruiting of *M. destruens*, the results of which are in the press, suggests that energy relationships play an important part in the processes leading to the production of perithecia.

Such a correlation between rate of respiration and the production of fruit bodies would be in agreement with results obtained with higher plants where reproduction is normally preceded by a high rate of respiration.

Thanks are due to the Editor and publishers of the *Annals of Botany* for permission to reproduce figures from my papers published in that Journal.

REFERENCES

ASTHANA, R. P. & HAWKER, L. E. (1936). The influence of certain fungi on the sporulation of *Melanospora destruens* Shear and of some other Ascomycetes. *Ann. Bot., Lond.*, L, 325–44.

BUSTON, H. W. & PRAMANIK, B. N. (1931 *a*). The accessory factor necessary for the growth of *Nematospora gossypii*. I. The chemical nature of the accessory factor. *Biochem. J.* XXV, 1656–70.

BUSTON, H. W. & PRAMANIK, B. N. (1931 *b*). II. The relation of the accessory factor to 'Bios'. *Biochem. J.* XXV, 1671–3.

BUSTON, H. W. & KASINATHAN, S. (1933). III. The preparation of concentrates of the second accessory factor. *Biochem. J.* XXVII, 1859–68.

GYÖRGY, P., ROSE, C. S., VIGNEAUD, V. DU, & MELVILLE, D. B. (1940). On the identity of vitamin H with biotin. *Science*, XCII, 62–3.

HAWKER, L. E. (1936). The effect of certain accessory substances on the sporulation of *Melanospora destruens* and of some other fungi. *Ann. Bot., Lond.*, L, 699–718.

HAWKER, L. E. (1938). Effect of growth substances on growth and fruiting of *Melanospora destruens*. *Nature*, CXLII, 1038.

HAWKER, L. E. (1939). The nature of the accessory growth factors influencing growth and fruiting of *Melanospora destruens* Shear, and of some other fungi. *Ann. Bot., Lond.*, N.S. III, 657–76.

HAWKER, L. E. (1942). The effect of vitamin B_1 on the concentration of glucose optimal for the fruiting of certain fungi. *Ann. Bot., Lond.*, N.S. VI, 631–6.

HAWKER, L. E. (1944). The effect of vitamin B_1 on the utilization of glucose by *Melanospora destruens* Shear. *Ann. Bot., Lond.*, N.S. VIII, 79–90.

KÖGL, F. & FRIES, N. (1937). Über den Einfluss von Biotin, Aneurin und Meso-Inosit auf das Wachstum verschiedener Pilzarten. *Z. physiol. Chem.* CCXLIX, 23–110.

KÖGL, F. & TÖNNIS, B. (1936). Über das Bios-Problem. Darstellung von krystallisierten Biotin aus Eigelb. *Z. physiol. Chem.* CCXLII, 43–73.

EXPLANATION OF PLATES

Perithecia where present can be seen with the aid of a hand lens as black dots. In Figs. 2–5 the *Melanospora* inoculum is the lower one. The Petri dishes used were 10·5 cm. in diameter.

PLATE VI

Fig. 1. *M. destruens* on medium A ten days after inoculation. No perithecia formed until fourteen days after inoculation.

Fig. 2. *M. destruens* and *Penicillium* sp. on medium A seven days after inoculation. Note ring of perithecia.

Fig. 3. *M. destruens* and *Monilia fructigena* on medium A twelve days after inoculation. Note ring of perithecia.

Fig. 4. *Melanospora destruens* and *Sclerotinia trifoliorum* on medium A, twelve days after inoculation. Note clear zone between colonies and perithecia formed where mycelium of *Melanospora destruens* reaches this zone.

Fig. 5. *M. destruens* and *Sphaeropsis malorum* on medium A twelve days after inoculation. Note perithecia of *Melanospora destruens* formed over area occupied by *Sphaeropsis malorum*. The hyphae of the two fungi intermingled.

Fig. 6. *Melanospora destruens* ten days after inoculation. Original medium in plate = medium A. Medium in segment, 0·1 strength of medium A.

PLATE VII

Fig. 7. *Melanospora destruens* ten days after inoculation. Original medium in plate = medium A. Medium in segment = ether-insoluble part of medium on which *Penicillium* sp. had been grown for seven days. Note numerous perithecia along line of junction.

Fig. 8. *Melanospora destruens* ten days after inoculation. Original medium in plate = medium A. Medium in segment = ether-soluble part of medium on which *Penicillium* sp. had been grown for seven days. Note resemblance to dilute medium in Fig. 6. A few perithecia have formed at periphery of segment but the dark patch along the line of junction is due to dark hyphae and not to perithecia.

Fig. 9. *Melanospora destruens* seven days after inoculation. Original medium in plate = medium A. Medium in segment = medium A + 0·2 % lentil extract. Note numerous perithecia on segment and absence of perithecia from rest of plate.

Fig. 10. *M. destruens* on medium A plus biotin (4γ/100 c.c. medium) ten days after inoculation. The hyphae have formed a thin layer over the surface of the medium. No perithecia formed.

Fig. 11. *M. destruens* on medium A plus aneurin (4γ/100 c.c. medium) ten days after inoculation. Illuminated from rear to show limits of colony and irregular growth. The dark objects are irregular knots of hyphae. No perithecia formed.

Fig. 12. *M. destruens* on medium A plus biotin plus aneurin ten days after inoculation. Numerous perithecia formed.

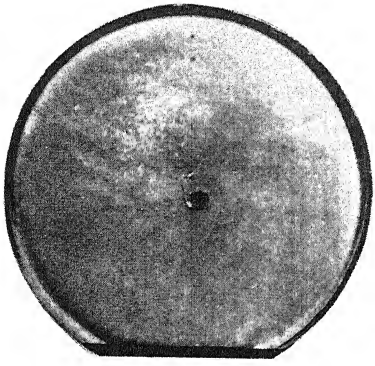


Fig. 1.

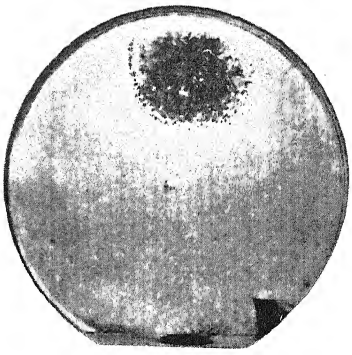


Fig. 2.

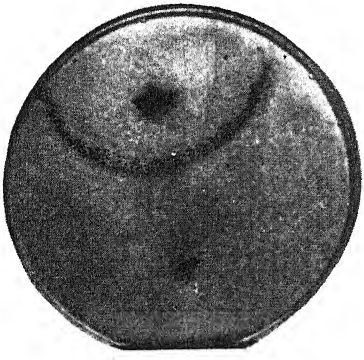


Fig. 3.

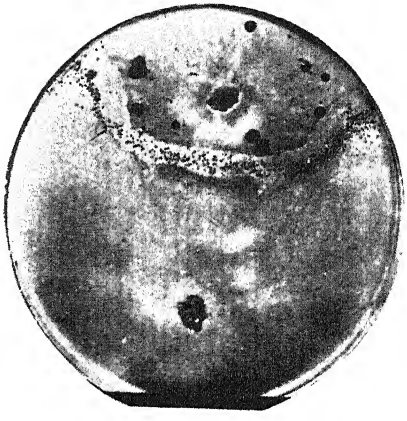


Fig. 4.

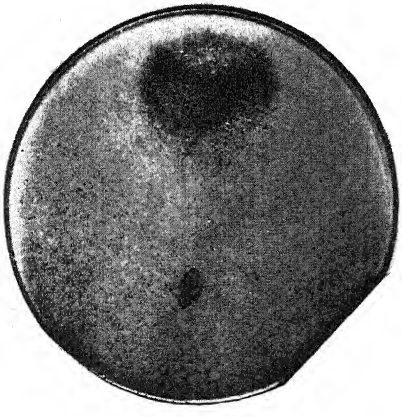


Fig. 5.

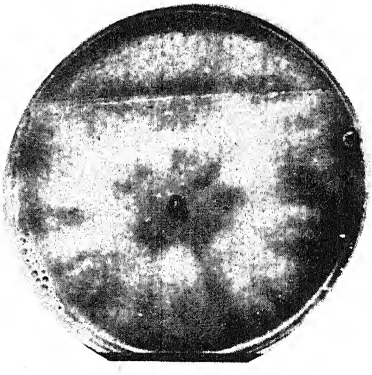


Fig. 6.

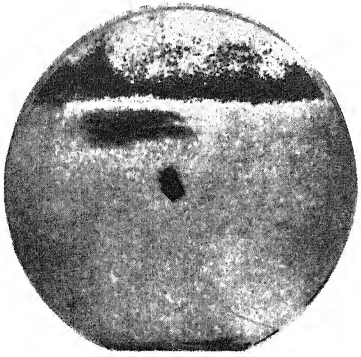


Fig. 7.

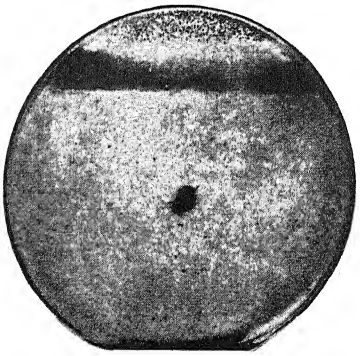


Fig. 8.

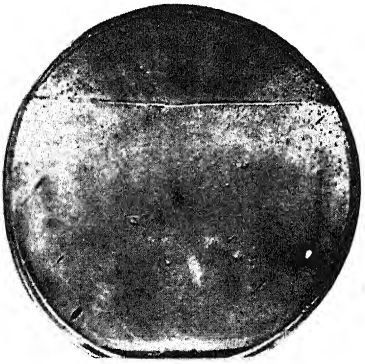


Fig. 9.

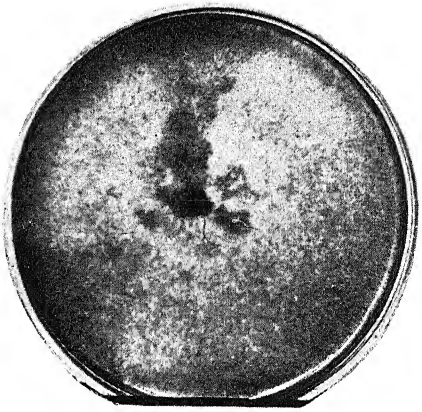


Fig. 10.

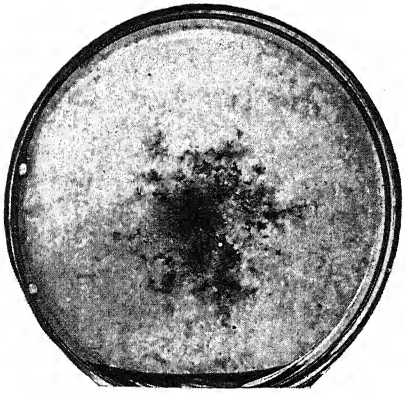


Fig. 11.

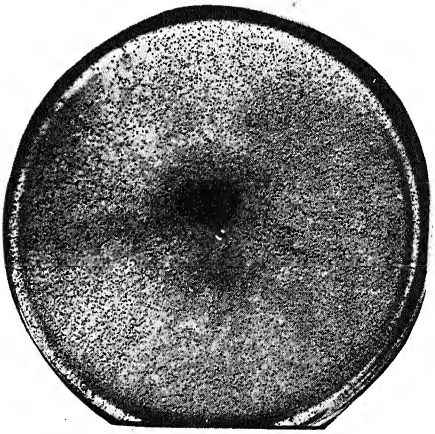


Fig. 12.

THE PROBLEM OF *CLADOSPORIUM HERBARUM*

By MARGARET A. BRETT
Northern Polytechnic, London

(With Plate VIII and 2 Text-figures)

Historical

The nature and relationships of *Cladosporium herbarum* have been a subject of controversy for upwards of fifty years, and in spite of the vast amount of investigation which has been carried out, there is still considerable uncertainty about the identity and behaviour of this fungus. In the past half century, the following three problems have been investigated:

(1) The possible connexion between *C. herbarum* Link and the form once known as *Dematium pullulans* de Bary.

To-day mycologists regard these as distinct fungi, and not different stages of the same fungus.

(2) The existence of the form-genus *Hormodendron* as a separate entity.

Some of the strains of *Cladosporium* were once said to have small unicellular spores and were known as *Hormodendron*, and the name *Cladosporium* was restricted to strains with septate spores, but as both these types of spore are said to be found in the same culture, modern opinion does not recognize the genus *Hormodendron* and the name becomes a synonym.

(3) The pathogenic nature of *Cladosporium herbarum*.

Some doubt exists about this, but the fungus is now believed to be usually saprophytic, although some species of *Cladosporium* are undoubtedly parasitic.

The work which led to these conclusions is summarized below:

(1) *The* Dematium—Cladosporium *relationship*. De Bary (1866) and Loew (1867) described *Dematium pullulans* as a septate-budding fungus, which ultimately forms bicellular chlamydospores or gemmae, but neither of these workers suggested that it was related to *Cladosporium*, although Loew called attention to certain similarities between them.

Laurent (1888) described a fungus which he termed *Dematium pullulans* bearing conidiophores of *Penicillium cladosporioides* Fres., i.e. *Hormodendron cladosporioides* Sacc. These were usually produced on non-budding hyphae, but on several occasions he observed them on budding mycelia, and one of these he figured. He also stated that he obtained *Dematium* from cultures of *Cladosporium*, particularly when these were exposed to the action of light. He concluded that *Cladosporium herbarum* and *Dematium pullulans* were the same fungus, *Dematium* being a weakened form ('un état affaibli') of *Cladosporium*. The view that they were related was accepted by Saccardo and others, and reproduced in works published subsequently.

From then onwards, however, there have been frequent criticisms of the accuracy of Laurent's results or observations. Jancewski (1892–4)

published three critical papers: he had, as he thought, repeated Laurent's experiments and obtained negative results. At one time he assigned a perfect stage to *Cladosporium*, viz. *Leptosphaeria tritici*; afterwards he corrected this, and stated that the perithecia of *Mycosphaerella tulasnei* belonged to *Cladosporium herbarum*. He still denied that any connexion existed between *Dematium* and *Cladosporium*. Again, Berlèse (1895) separated *Cladosporium* from a mixture of the two forms, and not being able to return to *Dematium* again, suggested Laurent's cultures were impure. As Laurent had admitted that his *Dematium* was originally obtained as a laboratory contaminant, it was thought probable that the *Cladosporium* might have arisen in a similar manner.

Planchon (1900), who was the first to make single spore cultures of this fungus, also severely criticized Laurent's work. He maintained that Laurent had an accidental mixture of the two forms, that his observations were inaccurate, due to the striking similarity between the vegetative mycelia of the two fungi, and that *Cladosporium herbarum*, which was usually united with *Dematium pullulans*, ought to be separated from it, and the two forms regarded as distinct fungi.

In spite of this sweeping statement, we find Delacroix and Maublanc (1909), and also Arnaud (1910), referring to the connexion existing between the two forms. Mention may here be made also of Massee (1898), who had obtained evidence confirming the relationship, and in his text-book stated that *Dematium pullulans* is the same fungus as *Hormodendron*.

Brooks and Hansford (1923), Hoggan (1923), and Bennett (1928 *a*), however, all failed to discover any genetic relationship between *Dematium pullulans* and any cladosporioid fungus, and Bennett described a perfect stage, *Anthostomella pullulans* (n.comb.) of which *Dematium pullulans* is the conidial form. This relationship, however, is questioned by Melin and Nannfeldt (1934).

Meanwhile *D. pullulans* de Bary had been transferred by Berkhout (1923) to the new genus *Pullularia*, as *P. pullulans* (de Bary & Loew) Berkh.

(2) *The existence and nature of the form genus* Hormodendron. To-day, mycologists regard *Hormodendron* as identical with, and practically indistinguishable from, *Cladosporium*. Earlier workers, however, observed certain differences between the two forms, though some suggested that they were connected with the nature of the medium, a rich medium encouraging profuse branching and budding.

Laurent (1888) observed that the *Hormodendron* form developed from the *Cladosporium*, and Costantin (1889) recorded a similar phenomenon. Jancewski (1892–4) also recognized two conidial forms, *Hormodendron* and *Cladosporium*, in association with the perithecia of *Mycosphaerella tulasnei*. Berlèse (1895) thought *Hormodendron* the true species type, and *Cladosporium* a secondary form. Planchon (1900) found that the principal axis of *Cladosporium* continues to lengthen after the production of conidia, while in *Hormodendron* it remains short, though he thought the mycelia and conidia were identical in the two forms. Schostakowitch (1895) found that the two types differed in phototropic response, roughness of conidial wall, and production of conidia in various concentrations of potassium nitrate.

Bancroft (1910) described *Hormodendron* as parasitic and *Cladosporium* as saprophytic, with what he thought was a regular alternation between the two forms. Mackie (1920) also found *Hormodendron* parasitic on wheat.

On the other hand, Bockmann (1933) stated that although certain of his strains of *Cladosporium herbarum* approximated to the *Hormodendron* type, he found no evidence of alternating development of the two spore forms, and regarded *Cladosporium* as a collective species, embracing the form *Hormodendron*.

Brooks and Hansford (1923) also stated that the difference between the two forms is connected merely with the degree and character of budding of the first-formed spores, and that they merge one into the other. Bennett (1928*b*) too, found that *H. cladosporioides* did not differ from *Cladosporium herbarum* 'morphologically, physiologically or biologically'. He did not think *Hormodendron* was parasitic, but admitted that it always appeared first when naturally infected material is incubated, though it could not be kept pure, and free from the *Cladosporium* type of conidiophore. According to him 'the bud spore form can be produced under a variety of controlled conditions, but cannot be developed as an entity either on dead or living matter'. And again '*Cladosporium* in its natural condition shows different kinds of growth simultaneously, the differences including colour of the organism in mass, both on the host and under the microscope, length, width, septation, colour and habit of the conidiophores, and size and shape of the conidia'.

The contradictory evidence relating to these three form genera has thus accumulated. It is probable that more than one form of budding fungus has in the past been termed *Dematium pullulans*, and that this is responsible for some of the confusion. But after an extensive survey of the literature and illustrations of *Cladosporium* together with conclusions drawn from the following practical investigation, I am of the opinion that there are other reasons for the discrepant accounts. One is that the cladosporioid type of conidiophore is found in more than one fungus, and investigators have not all been dealing with the same organism. Another is that the terms '*Cladosporium*' and '*Hormodendron*' have been used in different senses by different authors. For example, Bockmann's *Cladosporium* is a strain producing conidiophores which bear larger and fewer conidia per head, as compared with the *Hormodendron* strain; the two also differ in macroscopic appearance, e.g. in colour. Brooks and Hansford also regarded *Hormodendron* and *Cladosporium* as similar conidial strains.

On the other hand, the *Cladosporium* of Laurent, Costantin, and Bancroft is evidently a type of conidiophore produced on the same mycelium as *Hormodendron* conidiophores.

It is possible that there is also confusion in the use of the term *Hormodendron*, although experimental corroboration of this is needed. Costantin used it for the bud spores produced when *Cladosporium* spores germinate, as well as for the form which appears in the same culture as the *Cladosporium*. Yet he expressly stated that after several consecutive cultures had produced these two forms, the two types of spore produced growths of a different nature, one being a delicate form bearing minute *Hormodendron* conidiophores, the other a profusely sporulating bud spore form. In

Bancroft's fungus also, 'bud spores' were twice produced—when first culturing from infected material, and again on the germination of the *Cladosporium* spores which arose later in his cultures. He, however, regarded these as identical and both are termed *Hormodendron.* Is it certain that they are, in fact, the same?

The following preliminary account of certain cladosporioid forms, would appear to confirm the opinion that these facts are relevant to the confusion which exists in connexion with these form genera.

Experimental

Two cladosporioid fungi from the anthers of Ulex minor

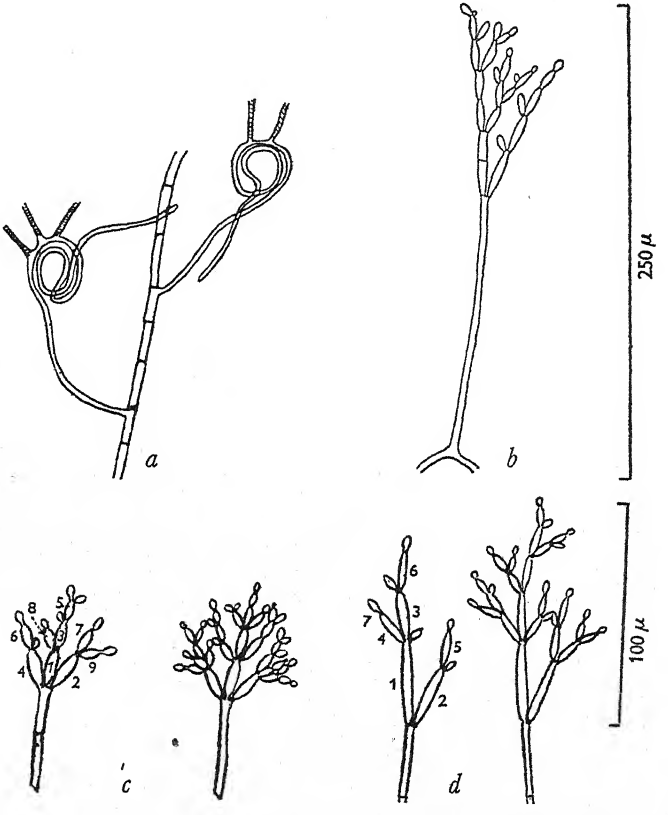
My interest in the form genera, of which an account has been given above, has been in the nature of a side line, and the work has been pursued only at infrequent intervals. Attempts were originally being made to isolate some comparatively rare smut fungi which infect the flowers of *Ulex minor* and form conidia on the surface of the anthers. Occasionally the spores of various imperfect forms, particularly yeast-like budding fungi, were present among the smut conidia. Several strains of a fungus resembling *Cladosporium herbarum* also occasionally appeared in cultures made from inocula taken from the anthers. The association of the budding fungus and the cladosporioid forms in the same host plant, might, it was realized, have no genetic significance, but it recalled past discussions and this led to consultation of the copious literature. The appearance of more than one type of conidial head, as described by Bennett (1928*b*), was noted when culturing the *Cladosporium*, and this suggested the first investigation with which this paper is mainly concerned, viz. an account of the various types of spore and spore-bearing structure observed. From the first fungus isolated two distinct conidial strains were obtained, a *Hormodendron* and a larger form resembling a *Cladosporium* which arose from it. The *Hormodendron* could not be prevented from producing this larger form, but once the latter was obtained, it could be maintained in culture.

In infected flowers of *Ulex minor*, a fungal layer of unicellular sprouts and budding smut conidia is formed on the surface of the anther, giving it a white powdery appearance. In some specimens, small rounded or somewhat angular thick-walled spores with a yellowish tinge to the wall were seen in the mass among the smut conidia. A number of these spores, when isolated from one infected anther, were found to give rise to mycelia producing conidiophores of the *Hormodendron* bud spore type. (It was noted that yeast-like budding cells were also present in great abundance on these anthers.)

The mycelium was septate, and 3–5 μ in diameter; when young the hyphae were hyaline, but as they matured became pale brown. The conidiophores were erect and not more than 20–30 μ in length. Owing to their extremely profuse production it was not possible to photograph them adequately, and diagrams do not convey an accurate picture of the dense mop-like heads of conidia which were produced. The bud spores tended to

hang together in overlapping clusters when disturbed, continuing to reproduce by budding on the medium.

A number of plate cultures of single spores from the anthers were made on a 1·0 % malt-agar medium. The central region of these was black, and the medium stained intensely. It was occupied by the *Hormodendron* mycelium producing short erect conidiophores. Surrounding this dark area, a paler zone was formed (Pl. VIII, fig. 1) in which aerial branches



Text-fig. 1. *a*, coiling of aerial branches in outer zone of cultures on Pl. VIII, fig. 1. Large conidiophores (cf. Text-fig. 1*b*) arose from coils. *b*, large conidiophore of '*Cladosporium*' type arising from coiled hypha. *c*, method of conidial formation in '*Hormodendron*' (diagrammatic). *d*, method of conidial formation in '*Cladosporium*' (diagrammatic).

developed, some of which showed intricate coiling. Among the conidial heads in this region, the stalks of which were variable in length, occasional larger stouter conidiophores arose, particularly in relation to the coils (Text-fig. 1 *a*). These bore larger conidia, the first formed of which were occasionally septate (Text-fig. 1 *b*). The surface of the culture in this area eventually became woolly, owing to the production of much aerial mycelium.

Many attempts were made to keep the original *Hormodendron* mycelium in culture by plating out single bud spores, but it was impossible to do so.

Subsequent cultures always contained a larger and different type of conidiophore produced in association with the coils, and sometimes also a more vigorous mycelium which swamped the original *Hormodendron*. When hyphal tips were taken from these more vigorous branches, pure mycelium of the second type was obtained bearing the *Cladosporium* type of conidiophore. The rate of development of the two forms was strikingly different, e.g. on the third day after sowing spores, the small *Hormodendron* mycelium was visible only under the microscope, while the *Cladosporium* had formed colonies about 3 mm. in diameter and was sporulating freely.

The *Hormodendron* bud spore mycelium, as obtained from the anthers, was therefore transitory and quickly gave rise to a strain producing a second conidial form. Should this be termed the *Cladosporium* form? It is believed to be so named by several authors but it may also be similar to the giant *Hormodendron* strain recorded by Jancewski.

The strain is evidently a vigorous mutant, and in view of the opportunities for nuclear association in the intricately coiled hyphae, it may conceivably be polyploid in nature.

The following differences could be observed between the two conidial forms:

(1) The coniodiphores were short and mainly erect in the *Hormodendron*, and longer, growing at various angles in the *Cladosporium*.

(2) The conidial heads were different in appearance owing to the manner in which the conidia were formed. In *Hormodendron*, three or four primary conidia were formed at the same level though not simultaneously. On these as axes, successively younger spores were formed in chains, branching occurring by the formation of a spore immediately behind the previous one (Text-fig. 1 *c*). The method was similar to that found in *Cladosporium*, but there were more branches at each node, and they were closer together. This produced a mop-like head of spores which, owing to the erect habit, was seen from above. In the *Cladosporium* one or two branches were formed at each node (more often one), but the greater elongation of the primary conidia gave the appearance of a central axis, which was often visible, owing to the angle of growth, and also because the branching was less dense, and the heads more lax in type (Text-fig. 1 *d*).

(3) *Hormodendron* conidia were smaller and more uniform in size than those of *Cladosporium*. The latter measured from 20 to 25 μ in length, down to about 4 μ. The largest in *Hormodendron* were not more than 15 μ.

(4) Pure *Hormodendron* colonies were black in colour, and stained the medium deeply. *Cladosporium* cultures were greyish green, and somewhat woolly. They also stained the medium, but less deeply than *Hormodendron* (Pl. VIII, fig. 2.)

It was noticed that in some subcultures, branches of a mycelium which was grown from a *Hormodendron* bud spore, widened and began to form the *Cladosporium* type of conidiophore. The two kinds of mycelium then grew out together, the *Cladosporium* above the *Hormodendron*. In other subcultures no *Hormodendron* whatever appeared to be formed, but it was noted that in these cases, the spores had been taken from old cultures. Young *Hormodendron* spores also produced relatively large colonies of pure *Hormodendron*,

suggesting that the age of the spores might be an important factor in the amount of this form produced in subsequent cultures. This would need further investigation, but the results recall Laurent's account of his fungus bearing the *Hormodendron* type of conidiophore, which he stated was reproduced if he sowed the spores at once, though later sowing resulted in a form of *Cladosporium herbarum*.

In the fungus obtained from this isolation there was, therefore, a constant tendency for a more stable and vigorous '*Cladosporium*' form to arise in culture from the *Hormodendron*. Once it was separated no return to the *Hormodendron* occurred, in contrast to the *Cladosporium* strains described by Bockmann in which sectors of *Hormodendron* developed.

Here then is one cladosporioid form, which, originally *Hormodendron*-like in appearance, constantly gives rise to a form or strain with characters associated with a *Cladosporium* type of fructification and habit of growth. It is difficult to be certain whether any previous workers have encountered exactly this phenomenon. Possibly Berlèse (1895), who thought *Hormodendron* the true species type, and *Cladosporium* a secondary development; possibly Planchon (1900), who described the principal axis of *Cladosporium* as continuing to lengthen after the production of conidia, while in *Hormodendron* it remained short; possibly Schostakowitch (1895), who found that the two types differed in phototropic response and certain other characters. Moreover, strains such as those described by Bockmann (1933) might presumably have arisen in some such manner as this; his record of *Hormodendron* sectors in *Cladosporium* cultures shows also that the reverse phenomenon may occur.

It was while attempting to obtain this same fungus from other infected anthers, that another cladosporioid type was isolated. Small, faintly coloured spores from these anthers first produced a minute mycelium, visible only under the microscope, which grew feebly in culture.

The hyphae were comparatively wide (6–7 μ), but tapered gradually as the mycelium ceased growth. The cells contained oil globules, and were irregular in form and size (Text-fig. 2 *a*). Occasionally a small kink or twist was visible in the mycelium. Close to the germinating spore, erect brown conidiophores producing bud spores of the *Hormodendron* type were first produced (Pl. VIII, fig. 3). Later, a comparatively few prostrate stalks, bearing heads of larger spores, were formed on the same mycelium (Pl. VIII, fig. 4 and Text-fig. 2 *b*). The first formed of these were two or three times septate, the later bicellular or unicellular. They were comparatively thick-walled and contained oil globules. This spore-bearing structure always lay flat on the medium, producing successive spores on short stalks, which persisted for some time as conspicuous necks.

It would appear from the various illustrations of this fungus, that a similar 'conidiophore' has been described and figured as the *Cladosporium* type by several authors. For example, Costantin (1889) obtained a fungus from *Haliotis*, which for several generations produced both *Hormodendron* and *Cladosporium*. It is also clear that Bancroft described a similar fungus obtained from various host plants, for he stated that

Hormodendron conidial heads were first produced on it, then on other fertile hyphae, conidia of the *Cladosporium* type were borne. These were typically one—and sometimes two or three times—septate. An examination of Costantin's figures shows that the *Cladosporium* spores produced on germination a mycelium bearing bud spores of the *Hormodendron* type on short stalks. According also to Bancroft, the *Cladosporium* spores on germination produced the *Hormodendron* type of spore again; this he regarded as an alternation of the two forms in the life cycle.

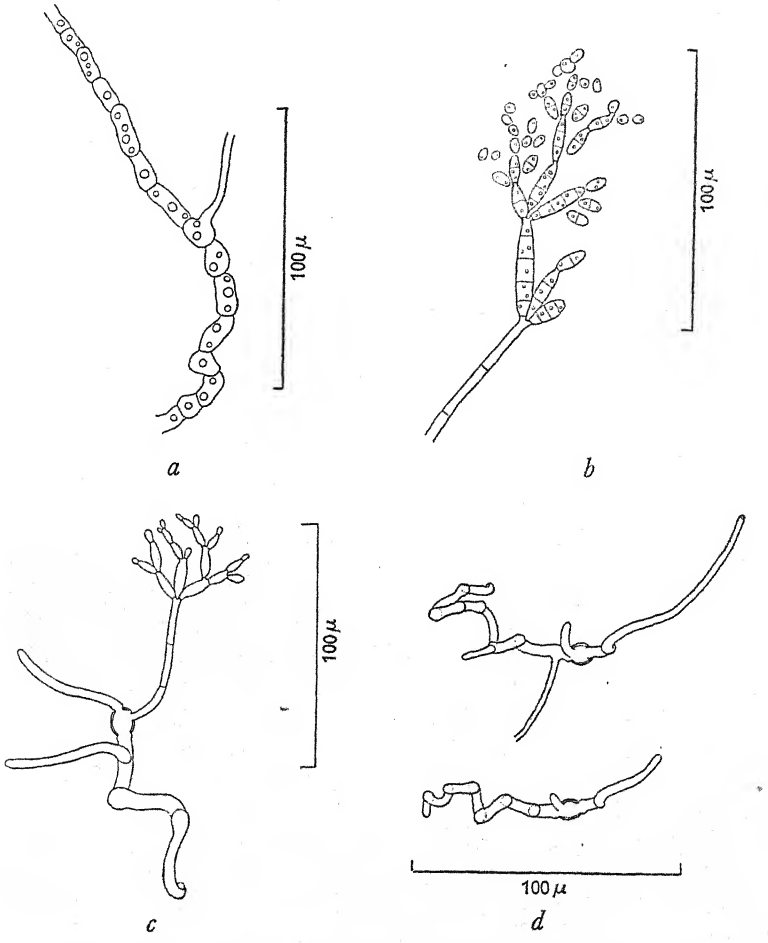
Now somewhat similar behaviour has been observed in connexion with the fungus under discussion. The prostrate spores, on germination, produce small chains of bud spores or minute *Hormodendron* conidiophores (sometimes in situ). When this fact was noted it appeared to be merely a corroboration of Bancroft's work, and the matter was pursued no further. Recently, however, the germination of these bud spores has been observed, and the method is found to be different from that of the *Hormodendron* spores on the erect heads. A single germ tube is formed and the mycelium to which it gives rise is very delicate (3–4 μ in diameter), profusely branching and bearing quite small *Hormodendron* conidiophores. Further investigation of the behaviour of this mycelium is now in progress.

If the spores from the erect heads are germinated, however, a purely conidial strain resembling a culture of the bud spore stage of *Cladosporium herbarum* is obtained, and the mycelium with its prostrate spores is entirely lost. Cultures of this conidial strain grow luxuriantly (Pl. VIII, fig. 5). They are grey-green in colour, with aerial branches forming a woolly surface growth, and brownish black submersed mycelium. When the spores germinate they form two or more characteristically 'kinked' germ tubes, 4–5 μ in diameter and sometimes also a conidiophore from the spore (Text-fig. 2*c*, *d*). The 'kinks', which occur in relation to the septa, are found in all the branches of the older mycelium, and appear to be the means by which much of it is carried below the surface of the medium.

A *Cladosporium* such as this (which has every appearance of the bud spore stage of *C. herbarum*) would therefore appear to be an isolated conidial stage grown from the conidia from the erect heads produced on a small mycelium bearing dimorphic spores. The conidiophores and conidia are similar to the erect heads from which the isolation was made. Such a bud spore stage, as several workers have suggested, is identical with, or embraces the *Hormodendron* form from which it is derived.

Reference must again be made here to the small mycelium bearing dimorphic spores, and obtained directly from the host plant. It was felt at the time that it might possibly be significant in the life history, but nothing further was demonstrated beyond the production of *Hormodendron* conidiophores from some of the spores and this, as already stated, had been observed by other workers. The mere fact of its existence seems to be important, however. A few workers, including Bancroft (1910), Lopriore (1892), Prillieux and Delacroix (1890), and Mackie (1920) described the *Hormodendron* stage of *Cladosporium herbarum* as parasitic. Bennett (1928*b*) found this view untenable, and stated that *Hormodendron* stages do not differ in parasitic capacity from normal *Cladosporium* forms. Is it possible that the

discovery of this feeble mycelium may reconcile the two views? Although no inoculation experiments have been carried out, it is a stage which appears to be parasitic on *Ulex minor*, since it was obtained from the anthers of unopened flowers. The *Cladosporium* bud spore conidial stage



Text-fig. 2. *a*, portion of mycelium which produces dimorphic spores. *b*, part of prostrate spore-bearing structure on same mycelium as *Hormodendron* conidiophores. *c*, germinating spore from erect head, showing kinked submersed hypha and conidiophore arising from spore. *d*, two stages in germination of conidia from erect heads on mycelium bearing dimorphic spores.

which can be isolated from it, however, may not be parasitic. Both points of view could be correct, therefore, for the two different stages.

This then is a second cladosporioid form from the same host plant, and it may, or may not, be related to the first. Workers on this type of fungus in the past have undoubtedly included Costantin and Bancroft, and probably

others, but it is possible that isolated conidial stages only have been handled by some investigators, in cases where no mention is made of the prostrate spore-bearing structure.

Note on a budding fungus from diseased pods of Ulex minor

During the time these fungi were in culture, no proof was ever obtained that they were connected with any budding form, although yeast-like cells were frequently present on the same anthers.

Quite recently, however, my interest has been revived in these form-genera. Some evidence has been obtained of a connexion between a budding fungus and *Hormodendron*-like conidiophores. The contents of pods of the same host plant had been examined for the presence of spore balls of the infecting smut. Those obtained from one of the pods were contaminated with other fungi, and the plate was discarded. Some weeks afterwards casual examination revealed a few thick-walled bicellular spores lying freely on the medium, with no indication of their origin or method of formation. These on germination yielded a budding fungus. As in the *Dematium pullulans* described by Laurent (1888), yeast-like colonies were first formed, and from these, radiating septate hyphae grew out, bearing clusters of thin-walled oval buds ($5–9 \times 3–4\mu$). These were formed near the septa and also terminally; they quickly became detached from the hypha, and, budding copiously, formed other yeast-like colonies. Occasionally among the buds (though not in all the colonies) small rounded cells with thicker walls were observed. A few of these gave rise in situ to minute stalks bearing a *Hormodendron* type of bud spore. In another culture small clusters of *Hormodendron* bud spores on minute conidiophores arose from branches connected with a submersed portion of the budding mycelium. The fungus is being further investigated, to determine if any connexion exists between it and the cladosporioid fungi from the anthers.

In concluding this preliminary note, it should be stated that the budding fungus does not appear to be identical with the *Pullularia pullulans* Berkh. isolated recently from Rye grass seed, a culture of which Dr Muskett has kindly sent for examination.

The little information that has so far been gathered about this fungus makes any concise summary, or comparison with earlier work impossible. The only two investigators who have claimed that a relationship exists between the budding fungus *Dematium pullulans* and a cladosporioid form are Laurent and Massee, and it is possible that this may be a similar budding form. To which of the anther cladosporioid forms it will prove to be related, if to either, is a subject for future investigation.

My sincere thanks are due to Mr W. F. Buck of the Plant Pathology Laboratory, Harpenden, for assistance in photographing the text-figures and for the excellent plates, and also to Dr J. Ramsbottom, for his encouragement at all times, and especially when the problem of *Cladosporium herbarum* has threatened, by its unsuspected complexity, to overwhelm the author.

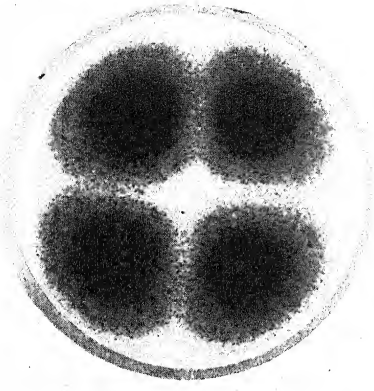


Fig. 1.

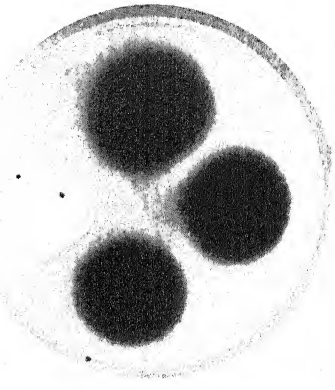


Fig. 2.

Fig. 3.

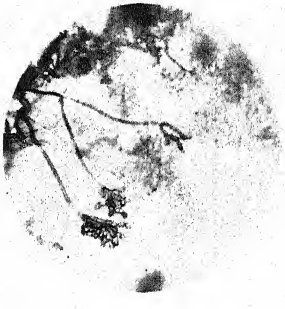


Fig. 4.

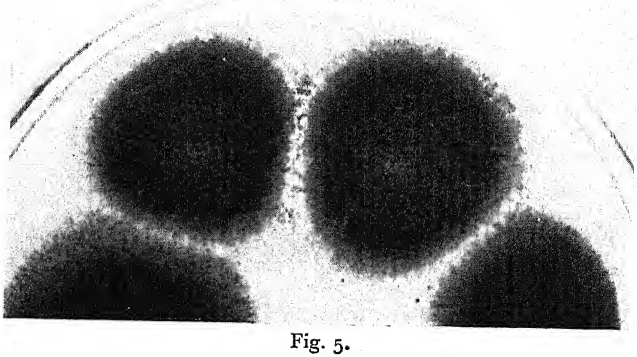


Fig. 5.

REFERENCES

ARNAUD, G. (1910). Contribution à l'étude des Fumagines. *Ann. Myc., Berl.*, VIII, 475.

BANCROFT, C. K. (1910). Researches on the life history of parasitic fungi. *Ann. Bot., Lond.*, XXIV, 359–71.

BENNETT, F. T. (1928 *a*). On *Dematium pullulans* and its ascigerous stage. *Ann. appl. Biol.* XV, 371–91.

BENNETT, F. T. (1928 *b*). On *Cladosporium herbarum*, the question of its parasitism, etc. *Ann. Appl. Biol.* XV, 191–211.

BERKHOUT, C. M. (1923). De Schimmelgeschlachten *Monilia*, *Oidium*, *Oospora*, en *Torula*. Dissert. Utrecht, 71 pp., 4 pl.

BERLÈSE, A. N. (1895). Première contribution à l'étude de la morphologie et de la biologie de *Cladosporium* et *Dematium*. *Bull. Soc. Myc. Fr.* XI, 34–72.

BOCKMANN, H. (1933). Die Schwärzepilze des Getreides unter besonderer Berücksichtigung ihrer Pathogenität und des Vorkommens von Rassen innerhalb der Gattungen *Cladosporium* Link und *Alternaria* Nees. *Angew. Bot.* XV, 308–21.

BROOKS, F. T. & HANSFORD, C. G. (1923). Mould growths upon cold store meat. *Trans. Brit. Myc. Soc.* VIII, 113–41.

COSTANTIN, M. J. (1889). Sur les variations des *Alternaria* et des *Cladosporium*. *Rev. Gén. Bot.* pp. 501–8.

DE BARY, A. (1866). *Morph. und Physiol. der Pilze, Flecht, und Myxom.* pp. 182–3.

DELACROIX & MAUBLANC (1909). *Maladies des Plantes cultivées*. Paris, p. 370.

HOGGAN, I. A. (1923). On *Dematium pullulans* de Bary. *Trans. Brit. Myc. Soc.* IX, 100–7.

JANCEWSKI, E. VON (1892). Sur le polymorphisme de *Cladosporium herbarum*. *Bull. Acad. Sci. Cracovie*, pp. 417–22.

JANCEWSKI, E. VON (1893). Les périthèces du *Cladosporium herbarum*. *Bull. Acad. Sci. Cracovie*, pp. 271–3.

JANCEWSKI, E. VON (1894). Recherches sur le *Cladosporium herbarum* et ses compagnons habituels sur les céreales. *Bull. Acad. Sci. Cracovie*, pp. 187–208.

LAURENT, M. E. (1888). Recherches sur le polymorphisme de *Cladosporium herbarum*. *Ann. Inst. Pasteur* II, 581–8.

LOEW, E. (1867). Über *Dematium pullulans* de Bary. *Jahrb. wiss. Bot.* VI, 467–75.

LOPRIORE, C. (1892). Black Mould (Schwärze) of Cereals. *Ber. deut. Bot. Ges.* X, 72–6.

MACKIE, W. W. (1920). Sooty Mold (*Hormodendron Cladosporioides*) on wheat. *Rep. California Agric. Exp. St.* (1920–21), p. 45.

MASSEE, G. (1898). Gummosis of *Prunus Japonica* Thunb. *Kew Bull.* pp. 321–5.

MELIN, E. & NANNFELDT, J. A. (1934). Researches into the blueing of ground wood-pulp. *Svenska Skogsvardsföreningens Tidskrift*, Häfte III, IV, 444.

PLANCHON, L. (1900). Influence de divers milieux chimiques sur quelques champignons du groupe des *Dematiées*. *Ann. Sci. Nat.*, Ser. II, VIII, 1–248.

PRILLIEUX & DELACROIX (1890). Parasitisme du *Cladosporium herbarum*. *Bull. Soc. Myc. Fr.* VI, 135–6.

SCHOSTAKOWITCH, W. (1895). Über die Bedingungen der Conidienbildung bei Russthaupilzen. *Flora*, LXXXI, 362–73.

EXPLANATION OF PLATE VIII

Fig. 1. Four single spore cultures of *Hormodendron* spores from anthers of *Ulex minor*.

Fig. 2. Three single spore cultures of larger *Cladosporium* spores obtained from *Hormodendron* cultures in Fig. 1.

Fig. 3. Erect *Hormodendron* type of conidiophore from mycelium producing dimorphic spores.

Fig. 4. Prostrate spore-bearing structures from mycelium producing dimorphic spores.

Fig. 5. Two single-spore cultures of conidia from erect *Hormodendron* heads, produced on mycelium bearing dimorphic spores.

(Figs. 1 and 2 illustrate the first, and Figs. 3–5 the second type of cladosporioid fungus.)

TAXONOMIC PROBLEMS IN HYMENOMYCETES

By ELSIE M. WAKEFIELD
Royal Botanic Gardens, Kew

The aims of taxonomy are twofold. In the first place, the task of the systematist is to collate, annotate, and so arrange the vast mass of material before him that it is possible, when he is faced with an unknown plant, to discover whether it has been previously described, and if so its correct name. The second and ultimate aim is so to classify plants that in the scheme of classification their natural relationships are expressed. The first is essentially the preparation of indexes and keys and is frankly utilitarian; the second is the ideal of taxonomy, but infinitely more difficult of attainment. Recognition of relationships is not reached merely by the study of existing descriptions: it requires detailed study of the plants themselves. Donk speaks of 'intuition', but what is intuition in this matter if not the result of wide and accurate knowledge of the plants with which one is dealing?

The classification of the higher fungi was originally based on gross morphology alone, modern microscopical and biological methods being unknown to the early mycologists. During the past thirty years the increasing use of the microscope in the study of the larger fungi, as well as the more primitive forms, has brought about a revolution in our ideas. Especially is this the case in the lower Basidiomycetes in which we now recognize microscopic characters which are of enormous value in the determination of species, and which sometimes serve also to indicate wider relationships. In a paper read at the Sixth International Botanical Congress held at Amsterdam in 1935 I discussed in some detail the question of differential characters in resupinate Hymenomycetes. Examples were given of the characters now used for the determination of these fungi, such as the various accessory hymenial organs—cystidia, setae, paraphyses and the like—the characters of the hyphae, and the nature and mode of development of the basidium. I pointed out the tendency among recent workers to regard certain of these characters, such as those of the basidium, as being of more fundamental importance than the configuration of the hymenium for the delimitation of genera and of higher groups. Since that date these new ideas have been pursued further, not only in the study of resupinate forms but in the Agaricales and Aphyllophorales generally, and it may not be inappropriate on this occasion to draw attention to some of the more recent work on these lines.

Our taxonomic problems fall into two groups: (1) the satisfactory delimitation of species, the fundamental units; and (2) the best method of grouping species to form genera, families and still higher taxonomic categories, and the relative importance of characters to be used for this purpose.

(1) Limits of species

I do not propose to go into the much-debated question of what is a species. The delimitation of a species may be a matter of convenience, and the species may not have the same value in different groups. In a genus such as *Clavaria* or *Mycena*, where there are well-defined microscopic as well as macroscopic characters, it is possible to recognize clear-cut morphological species. The position is very different in some other genera, such as *Agaricus* (*Psalliota*) and some of the resupinate Hymenomycetes, where the number of morphological characters available for specific distinction is few, and individuals vary in all directions. In *Corticium coronilla* v. H. & L., Miss Biggs (1937) has shown that under this name is included an assembly of forms varying in spore size, in hymenial form and in cultural characters. On cultural characters she was able to divide them into several distinct groups, yet all these groups are more closely related to one another than they are to any other of the species of fungi having a similar urniform basidium. To regard such groups as species would immeasurably increase the difficulties of species determination with no compensating advantage. It seems best, therefore, to regard *C. coronilla* as one complex species including many forms. The problem is analogous to that of biological races in the Rusts and Smuts. Similar difficulties are met with in all groups. Humphrey *et al.* (1931, 1932) studied in detail the species allied to *Ganoderma applanatum*, with the result that many described 'species' were reduced to forms or varieties of this one variable species. For some time I have been accumulating notes and drawings relating to the British species of the genus *Agaricus* (*Psalliota*). This is a genus which is clearly defined, but in which the species are very difficult to distinguish. Much of the difficulty no doubt is due to the fact that some of the characters are so evanescent. It is absolutely essential to see young specimens, and specimens which have not been handled too much, in order to make out for instance the characters of the veil, any special odour or colour change, and the microscopic characters of the gill-edge. Take, for instance, *Psalliota arvensis*, the Horse Mushroom, a species which is supposedly common and well known. In the old descriptions much stress was laid on the so-called 'double' annulus. This certainly distinguishes *P. arvensis* from *P. campestris*, but it does not distinguish *arvensis* from a number of other species. A ring of this type is found in *P. silvicola*, *P. augusta* and its allies, *P. haemorrhoidaria*, and to a less extent in *P. xanthoderma*. The 'double' character is in fact due merely to the persistence of remnants of the universal veil which can be traced more or less in all species of the genus. On examining specimens which have been collected or sent in as being *P. arvensis* one finds variation particularly in spore size. Turning to the literature for guidance, the result is as follows. Rea (1922), A. H. Smith (1939) and J. Schaeffer (1938) give for *arvensis* fairly large, elliptical spores (8–10 × 5–6 μ, 7–9 (–10) × 5–6 μ, and 7–8 × 5 μ respectively). On the other hand, Ricken (1915), Kauffmann (1918) and Lange (1939) give spores 6–7 × 3–4 μ, 6–7 × 4–4·5 μ, and 6·5–7 × 4·5 μ respectively. Hotson and Stuntz (1938) give 7–10·5 × 4–4·5 μ, that is, as

long as the spores seen by Rea, Smith and J. Schaeffer but slightly narrower. There are two very discrepant records of the spores of *arvensis*: Coker (1928) gives 4·4–5·5 × 3·3–4 μ, that is very small, while Miss Cayley (1936) gives spore measurements 8·9–11·1 × 6·4–6·7 μ for the plant which she at that time regarded as the Horse Mushroom. These aberrant records may be written off. The very small measurements of Coker suggest that he may have been dealing with *P. xanthoderma* or an allied form. Miss Cayley's plant I have since seen and recognized as the species which Bresadola figured as *P. villatica*. It is probably not the true *villatica* of Brondeau, nor is it *P. augusta*, to which Konrad and Maublanc referred this figure. Although it is a well-marked species and not uncommon, it is still not certain what it should be called. Considering the other spore measurements attributed to *P. arvensis* by the authors mentioned, these fall into two groups, and it seems obvious that at least two species have been confused under this name. The smaller spores agree very well with those of *P. silvicola*, and there does seem to be a robust form of *P. silvicola* which may be mistaken for *P. arvensis* if only macroscopic characters are considered. That this is the explanation of the small spores of some authors is confirmed by such statements as that of Ricken and Lange that *P. arvensis* occurs in woods, and of Kauffmann that it has a smell of anise; these characters are those of *P. silvicola*.

A. H. Smith (1939) considers that in the genus *Psalliota* spore measurements and spore shape are very constant. I do not think that spore size alone can be relied upon to distinguish species, though it is in many cases helpful, as in the example just cited. There is another character, already used to some extent by Lange, which may prove very helpful in diagnosing the species of *Psalliota*, namely the microscopic structure of the gill-edge. But again a warning is necessary. It is essential to make observations on perfectly fresh, young material, and always on comparable parts of the gills. Schaeffer (1938) attempted to use chemical reactions (the colour changes produced by certain acids and alkalies) in his work on *Psalliota*. In so far as I have tried these, I find that they may be useful in indicating *groups* of species, but they do not seem to be clear enough or precise enough to be used for the determination of the separate species. The only method of dealing with a genus such as *Psalliota* is to start *de novo*, observing and collecting notes as to every possible character, and how such characters are correlated. Species cannot be separated by one character alone, but only by considering the sum of all the characters.

(2) Groups higher than species

It is not easy to judge how much value is to be attributed to a particular character. Instances abound where insistence on one feature as a basis of classification has led to a grouping that in other respects appears unnatural. Thus there is what seems to be a completely unnatural separation of *Clavaria cinerea* and its allies from other species of *Clavaria*, if one insists on the importance of a cytological character—the direction of the spindle during nuclear division in the basidium. On this basis these species (as the

genus *Clavulina* Schroet.) are placed by Gäumann (1926) and Donk (1933) with *Craterellus* and *Cantharellus* in the order Cantharellales, while the remainder of the Clavariaceae fall into the usual position near the Polyporales. The genus *Tomentella* Pat. (=*Hypochnus* Fr. emend. Karst.) is a natural assemblage of loose resupinate forms having coloured hyphae and more or less coloured warted spores, obviously allied to *Thelephora* Ehrh. ex Fr. But the attempt to define the genus principally on the ornamentation of the spore wall has led to the inclusion of forms such as *Corticium sulphureum* Pers. non Fr. (=*Hypochnus fumosus* Fr., =*Phlebia vaga* Fr.) and *Corticium tulasnelloideum* v. Hoehn. & Litsch. (Rea, 1927), which in general structure are not allied to this group. A similar violence is done to natural relationships when all species having cystidia and slightly yellowish spores are included in the genus *Coniophorella*. The true Coniophorellas have large brown spores and protruding basidia like *Coniophora*. To put *Peniophora byssoidea*, because of its slightly coloured spores, with these is to strain the character of spore-colour beyond reasonable limits. Similarly with *Coniophora laeticolor* (Karst.) Karst.; Pilat (1933) included this in *Coniophorella*, but remarked 'Modo phylogenetico certe potius in genus *Peniophora* pertinat'. It is referred by Rogers and Jackson (1943) to *P. dryina* (B. & C.) Rogers & Jackson.

The genus *Peniophora* as defined to-day is, however, an assemblage of miscellaneous species which have in common only the possession of some kind of accessory hymenial organ coming under the rather vague category of cystidium. It has become a convenient repository for species which cannot at present be placed in more exactly defined genera. Similarly, *Corticium* is a residue of species having no marked accessory hymenial organ but differing greatly in general structure.

According to the particular character on which emphasis is laid the same species may be classified differently by different authors. Thus the old *Hydnum farinaceum* Pers. became *Odontia* for Bresadola, who used this genus for resupinate Hydnums in general. For Bourdot it was *Grandinia*, because he limited the genus *Odontia* to species having cystidia. Donk did not change the name but dealt with it in the group Humicola of *Corticium*, where undoubtedly, apart from hymenial configuration, its affinities seem to lie. *C. polygonium* (Pers.) Fr. is a *Gloeocystidium* for von Höhnel (1908*a*), *Peniophora* for Bourdot and Donk, and *Aleurodiscus* for von Höhnel later (1908*b*) and Pilat (1926). Actually its affinities seem to be with the section *Coloratae* of *Peniophora*, to which group might also be added *Stereum pini* and *S. rufum*. *Peniophora hydnoides* Cooke & Massee and *Odontia hydnoides* v. Höhn. & Litsch. (=*O. conspersa* Bres.) are the same fungus differently named according to the degree of development of hymenial configuration. A bright yellow fungus which occurs in tropical and subtropical regions has a form which was described as *Corticium chrysocreas* B. & C. from the southern United States and as *C. archeri* Berk. from Tasmania. The same fungus, with a development of hymenial spines, received the names of *Kneiffia wrightii* B. & C. from Cuba and *K. chromoplumbea* B. & Br. from Ceylon. In other cases, species have been described in different genera and different families, which although not identical are obviously related as to

structure. Such series of related species are to be found in many groups. For instance, in the Polyporaceae examples are the series beginning with *Trametes hydnoides* Fr., including *Hexagona hystrix* (Cooke) Har. & Pat. and ending at the other extreme with *H. hirta* (Pal.) Fr.; or such a group as *Polystictus floccosus* (Jungh.) Fr., *Trametes cristata* Cooke, *Hexagona dybowskii* Pat. and their allies. There are many such series, which cannot fail to strike the mycologist who has frequently to work through the specimens in a general herbarium.

In the paper given at Amsterdam I drew attention to the work of Donk (1931, 1933) and of Rogers (1934) in pointing out such relationships among resupinate species, and in particular I mentioned the suggestion that the urniform basidium, which is found in certain species of *Corticium*, *Grandinia* and *Poria*, and also in *Sistotrema confluens* the type of the genus *Sistotrema*, is a character of fundamental importance indicating common descent of these forms. Later Rogers (1935) adopted a suggestion made by Donk, and included all these forms in the genus *Sistotrema*, which was therefore emended, and in fact became a new genus based on the nature of the basidium and not as heretofore on hymenial characters. Later still, Rogers (1943, 1944) modified this concept to the extent of retaining *Sistotrema* for pileate species only, and adopting the generic name *Trechyspora* Karst. for all resupinate species having the urniform basidium. *Trechyspora* was based on a poroid species, *Poria onusta* (Karst.) Bres. Rogers's revised *Trechyspora* therefore includes species of *Corticium*, *Grandinia* and *Poria* of the old classifications. The decision to separate *Sistotrema* does not seem logical under the circumstances, but was done as a matter of convenience. That at once raises the question of what we are aiming at in our classification. A classification there must be, otherwise there is chaos. In the first instance we must have a classification that is convenient, that is, one by means of which we can correctly identify our plants. For this purpose artificial systems may have their uses. As stated already, the ultimate aim of our classification, however, is to express, if possible, natural relationships. As our President emphasized in his address, it is not to be expected at this stage in evolution that existing organisms can be arranged to form one phylogenetic sequence. We can only get hints, here and there, of what may have been the history of certain groups. The difficulty seems to be to assess the importance to be given to characters which are agreed to have fundamental value, while at the same time retaining the practical nature of our systems of classifications. Most mycologists will agree as to the importance of the urniform basidium, which can be traced as a development from the basidium found in some of the Heterobasidiae. It is, however, open to question whether it is really helpful to include all species with such a basidium, or even all the resupinate species, in one genus. It might be better to regard such a basidium as a characteristic of a family, comparable with the peculiar basidium of the Tulasnellaceae, and within such a family to distinguish genera in other ways.

There have been various schemes of classification of the Hymenomycetes proposed in modern times to replace the Friesian system. Patouillard's '*Essai Taxonomique*' (1900) was the first and greatest attempt

to elaborate a system of classification that would seem more natural than that based solely on hymenial configuration. Patouillard's work has been the basis of most modern treatments of the Aphyllophorales generally. Bourdot and Galzin (1927), Donk (1931, 1933) and D. P. Rogers (1935, 1943, 1944) especially have elaborated new ideas with regard to the resupinate Hymenomycetes, and have shown great feeling for natural affinities. In recent years a great deal has been written about the Agaricales and ideas as to their relationships and classification are undergoing profound changes. Of these I do not propose to speak. I would like, however, in conclusion to draw attention to some modern work on the Polyporales.

In this country we have been very conservative and, on the whole, still adhere to the Saccardo classification, which was based on Cooke's *Praecursores ad Monographia Polypororum* (1855–6), and was merely an expansion of the work of Fries. When working through the Polyporaceae of the New York Botanic Garden herbarium, some twenty years ago, I was impressed with the fact that there seemed to be a good deal of logic in some of the divisions made by Murrill (1907–8). Murrill followed Karsten (1881) in dividing up the Polyporaceae into many small genera based on anatomical and spore characters. He used many of Karsten's names and added some others. Unfortunately, some of his genera were not well founded, and the system was not clear; consequently it fell into disrepute. The classification of Patouillard was much more clear, and has become familiar to us through the great work of Bourdot and Galzin, *Hyménomycètes de France* (1928). Bourdot and Galzin, however, did not follow Patouillard all the way. They retained *Hymenochaete*, for instance, in the subtribe Corticieae along with *Stereum*, *Corticium*, etc., whereas Patouillard included *Hymenochaete* and *Hydnochaete* in his series Igniarieae of the Polypores, that is along with all the brown Polyporaceae having setae in the hymenium. As compared with the older classifications we note that *Polyporus* is restricted to fleshy stipitate species with white flesh, while *Polystictus* and *Fomes* are abandoned. *Daedalea* is used only for *D. biennis*, and *D. quercina* is included in *Lenzites*.

Donk (1933) proposed a somewhat different grouping, with the Polyporoideae divided into four main groups, Polyporoideae, Hymenochaetoideae, Ganodermoideae and Fistulinoideae. Some of the genera proposed by Karsten (1879, 1881) and Quélet (1886) are retained and some new ones proposed. *Fomes* is retained for species allied to *F. fomentarius*, and *Polystictus* for stipitate Polypores of the *Polyporus perennis* group, as restricted by Ames (1913). In Hymenochaetoideae we have again the suggestion that the *Hymenochaete* seta and reaction to potassium hydroxide is of more importance than the form of the hymenium, but 'on practical grounds', as he himself says, Donk deals with *Hymenochaete*, also with *Asterostroma* and *Asterodon*, along with other genera which have a smooth or a toothed hymenium.

The treatment of the Polypores by Pilat, in the *Atlas des Champignons de l'Europe* vol. III (1938), is not unlike that of Donk, but differs in taking out the Fistulinaceae as a family equivalent to Boletaceae and Polyporaceae.

The Boletaceae are now of course generally regarded as not being related to the true Polypores but rather to the Agaricales. Under Polyporaceae Pilat, like Donk, has the tribes Polyporoideae, Ganodermoideae and Hymenochaetoideae. His treatment of the genera differs in the Polyporoideae, where he has sixteen genera instead of Donk's twenty-one. The most outstanding feature is that under *Trametes*, which he emends, he includes all the genera having one layer of tubes and homogeneous context, that is, besides the old *Trametes* also *Coriolus* Quél. (=most of our present *Polystictus*), *Daedalea* Pers., *Lenzites* Fr., *Hexagona* Fr., *Hirschioporus* Donk (founded on *Polystictus abietinus* (Dicks. ex Fr.) Fr.) and *Irpex* Fr. To anyone accustomed to working with tropical species, or even with polymorphic European forms like the series *Trametes rubescens—Daedalea confragosa—Lenzites tricolor*, the fusion has much to commend it. The alternative is to create many small new genera within this group, which would add further complication.

Lastly, I would mention the latest scheme of classification of the Polyporaceae, proposed by Bondarzew and Singer (1941), where a more extended attempt is made to express what may be called 'criss-cross' relationships and interweaving of characters. Bondarzew and Singer divide the Homobasidial Aphyllophorales into six suborders, namely Phylacteriineae, Clavariineae, Polyporineae, Cyphellineae, Corticiineae and Boletineae. Excluding the Boletineae, which need no explanation, poroid forms are distributed among the remaining suborders as follows:

(1) *Boletopsis* Fayod, based on *Polyporus leucomelas* with its angularly warted spores, is placed in the Phylacteriineae, that is, in the alliance of *Thelephora*, *Tomentella* and *Hydnums* with coloured warted spores.

(2) In the Clavariineae are placed certain fleshy species with hyaline spores, including *Polyporus giganteus* and its allies, *P. berkeleyi*, *P. ovinus*, *P. cristatus*, etc., that is roughly the section *Merisma* of older classifications. The criterion appears to be the suggested development of such a fruit-body from the *Clavaria* type.

(3) *Fistulina* and *Porothelium* are placed with the Cyphellineae, the distinct tubes being regarded as more akin to the cups of *Cyphella* than to the tubes of a true Polypore. Also in this suborder Singer later (1945) placed species of *Campanella*, *Favolaschia* and *Leptotus* (the family Leptotaceae of Maire).

(4) With the Corticiineae are placed the genera *Sistotrema*, *Trechyspora* and *Byssocorticium*, all of which are defined as including poroid forms, while the Meruliaceae form a second family of this suborder.

(5) The Polyporineae includes the family Polyporaceae in the limited sense, with the great majority of the Polypores. Bondarzew and Singer divide these into no less than fifty-three genera, arranged in five subfamilies.

A critical examination of these genera and of the distinguishing characters given convinces one that much of the work is not based on practical acquaintance with the plants themselves, but has been compiled from indications given by other authors. Sometimes these indications have not been rightly understood, as when *Trechyspora* is said to be distinguished from *Sistotrema* by having normal instead of urniform basidia. Some of the

new genera proposed appear to be erected on trivial distinctions; for instance, *Pseudotrametes* B.-S. with type species *P. gibbosa* (Pers.) B.-S. is distinguished from *Trametes* (type taken as *T. suaveolens*) chiefly by having smaller spores and no odour. The whole scheme gives the impression of an attempt to force species and genera into a preconceived plan, and seems likely only to create more confusion.

Before we can attain to a clear and workable classification of the Polyporaceae, and of other groups of the Basidiomycetes, we need a much more detailed knowledge than we at present have of hymenial structure and development, and of the minute anatomy of the fruit-bodies. As examples of the kind of work required I would point to that of Humphrey (1931, 1932) on the *Ganoderma applanatum* complex and of Haddow (1931) on the species of *Ganoderma* allied to *G. lucidum*; further to the anatomical work carried out by Corner (1932) on *Polystictus xanthopus* and on one of the brown species of *Fomes*, *F. laevigatus* Corner (1932 *a*), and the work of Kurt Lohwag (1940) and others on the structure of the outer layer of the pileus. Only by such careful work, extended to as many species and genera as possible, can we hope to build up a true picture of relationships and to attain a classification which will enable us to name our fungi with some degree of certainty.

REFERENCES

Ames, A. (1913). A consideration of structure in relation to genera of the Polyporaceae. *Ann. Mycol.* xi, 245.

Biggs, R. (1937). The species concept in *Corticium coronilla*. *Mycologia*, xxix, 686–705.

Bondarzew, A. & Singer, R. (1941). Zur Systematik der Polyporaceen. *Ann. Mycol.* xxxix, 43–65.

Bourdot, H. & Galzin, A. (1927). *Hyménomycètes de France.*

Cayley, D. M. (1936). Spores and spore germination in wild and cultivated mushrooms. *Trans. Brit. Mycol. Soc.* xx, 229.

Coker, W. C. (1928). The Chapel Hill species of the genus *Psalliota*. *J. Elisha Mitchell Sci. Soc.* xliii, 253.

Cooke, M. C. (1885–6). Praecursores ad Monographia Polypororum. *Grevillea*, xiii, 80, 114; xiv, 17, 78, 109.

Corner, E. J. H. (1932). The fruit-body of *Polystictus xanthopus* Fr. *Ann. Bot., Lond.*, xlvi, 71–111.

Corner, E. J. H. (1932 *a*). A *Fomes* with two systems of hyphae. *Trans. Brit. Mycol. Soc.* xvii, 51–81.

Donk, M. A. (1931). Revisie van der nederlandse Heterobasidiomycetae en Homobasidiomycetae—Aphyllophoraceae I. *Med. Nederl. Mycol. Ver.* xviii, xix, xx, 67–200.

Donk, M. A. (1933). Revision der niederlandschen Homobasidiomycetae—Aphyllophoraceae II. *Med. Bot. Mus. Herb. Rijks Univ. Utrecht*, No. 9, 1–261.

Gäumann, E. (1926). *Vergleichende Morphologie der Pilze*, pp. 499–500.

Haddow, W. R. (1931). Studies in *Ganoderma*. *J. Arnold Arbor.* xii, 25–46.

Hotson, J. W. & Stuntz, D. E. (1938). The genus *Agaricus* in western Washington. *Mycologia*, xxx, 214.

Humphrey, C. J. & Leus, S. (1931). A partial revision of the *Ganoderma applanatum* group, with particular reference to its oriental variants. *Philipp. J. Sci.* xlv, 483–567.

Humphrey, C. J. & Leus-Palo, S. (1932). Supplementary notes on the *Ganoderma applanatum* group. *Philipp. J. Sci.* xlix, 159–78.

Karsten, P. A. (1879). Symbolae ad Mycologiam fennicam VI. *Med. Soc. Faun. Fl. Fenn.* v, 37–40.

Karsten, P. A. (1881). Enumeratio Boletinearum et Polyporearum Fennicarum. *Rev. Mycol.* iii, 16–19.

KAUFFMANN, C. H. (1918). *The Agraicaceae of Michigan*, p. 236.
LANGE, J. (1939). *Flora Agaricina Danica*, IV, 57.
LOHWAG, KURT (1940). Zur Anatomie des Deckgeflechtes der Polyporaceen. *Ann. Mycol.* XXXVIII, 401–50.
MURRILL, W. A. (1907–1908). *North American Flora*, IX, 1–131.
PATOUILLARD, N. (1900). *Essai taxonomique sur les familles et les genres des Hyménomycètes.*
PILAT, A. (1926). Monographie der mitteleuropalschen Aleurodiscineen. *Ann. Mycol.* XXIV, 222.
PILAT, A. (1933). Additamenta ad floram Sibiriae Asiaeque. *Bull. Soc. Mycol. Fr.* XLIX, 296–8.
PILAT, A. (1938). *Atlas des Champignons de l'Europe, III, Polyporaceae.*
QUÉLET, L. (1886). *Enchiridion Fungorum.*
REA, CARLETON (1922). *British Basidiomycetae*, p. 84.
REA, CARLETON (1927). Appendix to British Basidiomycetae. *Trans. Brit. Mycol. Soc.* XII, 222.
RICKEN, A. (1915). *Die Blätterpilze*, p. 237.
ROGERS, D. P. (1934). The Basidium. *Univ. Iowa Studies*, XVI, 160–81.
ROGERS, D. P. (1935). Notes on the lower Basidiomycetes. *Univ. Iowa Studies*, XVII, 19–23.
ROGERS, D. P. (1943). The genus *Pellicularia*. *Farlowia*, I, 95–118.
ROGERS, D. P. (1944). The genera *Trechispora* and *Galzinia* (Thelephoraceae). *Mycologia*, XXXVI, 70–103.
ROGERS, D. P. & JACKSON, H. S. (1943). Notes on the synonymy of some North American Thelephoraceae and other resupinates. *Farlowia*, I, 263–336.
SCHAEFFER, J. (1938). Beitrag zur *Psalliota* Forschung. *Ann. Mycol.* XXXVI, 78.
SINGER, R. (1945). The *Laschia* complex. *Lloydia*, VIII, 170–230.
SMITH, A. H. (1939). Studies in the genus *Agaricus*. *Pap. Mich. Acad. Sci. Arts & Lett.* XXV, 116.
VON HÖHNEL, F. & LITSCHAUER, V. (1908*a*). Oesterreichische Corticieen. *Wiesner Festschrift*, p. 69.
VON HÖHNEL, F. & LITSCHAUER, V. (1908*b*). Norddeutsche Corticieen. *Oesterr. bot. Zeitschr.* LVIII, 475.

Since this short review was written, the anatomical work on Polyporaceae initiated by Corner has been carried a stage further by G. H. Cunningham (1947). He has worked out a key for the identification of about one hundred species of New Zealand Polyporaceae, using the characters provided by hyphal and basidial types.

CUNNINGHAM, G. H. (1947). Notes on classification of the Polyporaceae. *N.Z. J. Sci. Tech.* XXVIII, no. 4 (Sec. A), 238–51.

PHYLOGENY AND NATURAL CLASSIFICATION OF MACRO-FUNGI

By ROGER HEIM
Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle, Paris

(With 19 Text-figures)

For almost a century, Elias Fries's classification, and the principles which gave rise to it, have governed systematic mycology. They have played a fundamental part in the history and progress of the science. They have greatly eased the work of mycologists by enabling them to find their way among the numerous forms of macrofungi.

However, the principles based on the shape and appearance of the hymenium, which served the eminent Swedish mycologist, have been subject to modification and well-based criticisms in the past fifty years.

Nowadays, the arrangement of groups proposed by Fries and his followers is no longer satisfactory, at least in certain important particulars.

It is not my intention, to-day, to give a history of the new tendencies associated particularly with the names of Patouillard and Fayod. I propose merely to discuss some very recent results which provide weighty reasons for the construction of a more natural classification, taking into account essential affinities, i.e. those which tend to give it a reasonable phylogenetic interpretation.

1. Asterosporales

Bucholtz (1902 *b*) was the first to consider the relationships between *Lactarius* and *Russula* on the one hand, and between certain Hypogeae on the other (*Arcangeliella*, *Martellia* and subepigeous Gasteromycetes with chambered hymenium (*Elasmomyces*)). G. Malençon (1931) extended the conception. Laying stress on the particular characters of the flesh (groups of spherocysts enclosed in bundles of connective hyphae, lactiferous hyphae, etc.), and the presence around the spores of an amyloid perispore which breaks into warts often joined by striae of the same glucocidal material, he proposed to attach to the Lactario-Russulae nine genera dispersed among the Phallaceae, Secotiaceae, Hymenogastraceae. In this way he defined as a natural series, the so-called Asterosporae, comprising Russulaceae (epigeous, agaricoid, with hymenium often lamellated), and Asterogastraceae (subepigeous or epigeous, with chambered hymenium, closed or becoming closed). As early as 1934, we adopted this view, which supports the general idea of natural affinities existing between agaricoid and gasteroid forms, contrary to the Friesian concept which opposes and separates them. The discovery of new asterosporic forms during our expeditions in Madagascar and tropical Africa, enabled us to complete the scheme already so well developed by Malençon.

The very notion of a 'série naturelle des Astérosporés' demands that the

most simple phylogenetic forms should be determined. This fact, not dealt with by Malençon, had already been considered by Fayod, who believed that he had recognized the relatively primitive forms from which *Lactarius* and *Russula* arose. Owing to the discovery of the African and Madagascan species, we were able to construct the phylogeny of the Asterosporae on a general plan by associating them with relatively simple forms.

In the first place we specified the significance of compact forms of which Russulae compactae (*Lactarioides* or *delica* group, *Nigricantes* or *nigricans* group) and the Lactarii compacti (*Piperati* or *piperatus* group, *Dulces* or *volemus* group) constitute the components. The discovery of *Lactarius rubroviolasceus* in the eastern coastal forest of Madagascar brought a new element in support of bringing together simple forms of Lactarii and Russulae: this *Lactarius* has the essential characters of *Russula adusta* and *R. nigricans*, from which it differs especially in its abundant and watery latex. Later on the discovery at the Ivory Coast of *Lactarius melanogalus* Heim, extremely closely related to *Venolactarius adhaerens* Heim, except for the taste of the milk and the insertion of the gills on the stem, and *Russula fragilissima* Heim, physiognomically very near to *Lactarius gymnocarpus* Heim, and like it, having decurrent gills, has strengthened the view that: *the Compactae group represents a mixed Lactario-Russulae section*, showing a natural interweaving of russuloid and lactarioid forms with or without resinoid latex. 'A l'origine se perd le critère distinctif sur lequel est basée l'opposition entre les deux genres friesiens.'

Another argument in favour of this theory was provided by the discovery of the Russulas which we called Archaeinae, apparently even more primitive, with thick and distant, decurrent gills, like those of *Hygrophorus* (which is insufficient to warrant Fayod's hypothesis, difficult to uphold, that Lactario-Russulae were derived from *Hygrophorus*).

Thus, save for this common origin, *Russula* and *Lactarius* evolved independently, showing a very different range in their development, *Russula*, with varied and multiple types, forming the flourishing branch whose end terms (Chameleontinae, Urentinae), composing the *Russularia* section, show themselves much more advanced than the corresponding *Lactarius* forms.

Thus the apparently simplest representatives of the Asterosporae series revealed themselves in all probability as well-defined forms. Moreover, the very direction of evolution in both genera until its termination in Russulariae chromosporae, seemed apparent. It remained to give a more precise position in the series to the hypogeous Asterosporae well-defined by Malençon. A particular point which still requires elucidation is this: if the relationship between Hydnangiae and Russulae was clearly shown by Malençon, are we to consider these hypogeous forms as simple, even primitive, or on the contrary, *degraded*?

The simultaneous discovery of annulated Russulas, in the Congo by Madame Goossens (species described by Beeli), and in Madagascar, afterwards in West Africa and recently in the Cameroons by myself, and the embryological and anatomical study of this material, led to the filling of a gap which existed in the chain of the Asterosporales.

We (1940) demonstrated the pseudo-angiocarpic origin of these annulated Russulas just as Kühner (1926) did in the annulated Boleti. In both the annulus is a *secondary* formation resulting from a mechanical phenomenon rather than a strictly anatomical one; angiocarpy is only apparent, transitory; the annulus is not specifically constant either in its appearance (simple or double) or in its localization (peripileic or peripedicular) or even in its occurrence. The African genus *Lactariopsis* P. Henn., also pseudo-angiocarpic (Fig. 1), shows the same kind of annulated *Lactarius*. (We have found this genus again in Madagascar.)

The thorough study of the Madagascan Russulae revealed non-annulated forms (Aureotactinae, Heliochrominae), however, nearer to one or the other of the two groups of annulated *Russula* (*annulata* and *radicans*—the latter showing a rooting stipe (Fig. 5)) than they are to one another.

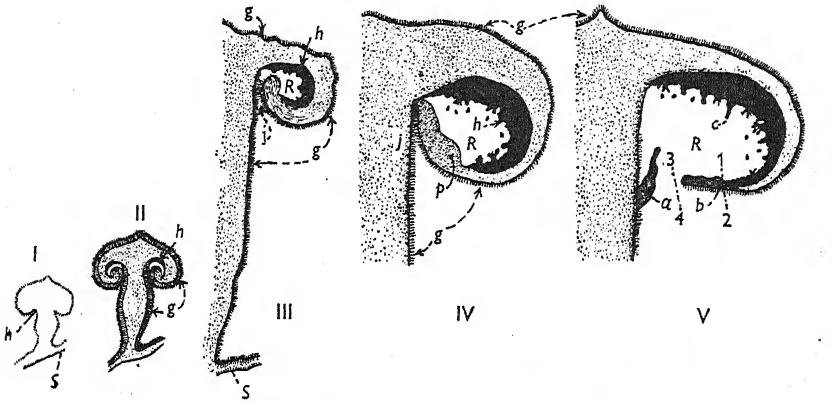


Fig. 1. Reconstitution of the pseudo-angiocarpic development of *Lactariopsis pandani* (schematized longitudinal sections). *R*, hymenial chamber; *g*, blematogeneous or general veil with thick-walled radial hyphae; *h*, hymenium; *S*, basal subiculum; *j*, junction of two pileic and peripedicular parts of the general veil; *p*, partial veil; *a*, annulus of the stipe; *b*, appendiculum resulting from the rupture of the partial veil according to iii, iv; the rupture according to i, ii would leave all the partial veil around the stipe in the form of a membraneous annulus (R. Heim, 1937).

Relying on the sum of the morphological, micrographical and biochemical characters, we defined a large section, including all annulated forms, under the name of Pellicullariae, though these constituted only a part of the whole.

Thus the same agaricoid genus, *Russula* or *Lactarius*, includes side by side gymnocarpic species (non-annulated) and pseudo-angiocarpic (annulated). The near relationship of these to gasteroid forms is supported by the new fact that the gasteroid genus *Elasmomyces* includes, according to Bucholtz, a species (*E. krjukowensis* Buch.) primitively subgymnocarpic, then angiocarpic (namely pseudo-angiocarpic), and another species (*E. mattirolianus* (Fig. 4)) strictly angiocarpic. The spores of *Lactarius adhaerens* recall strongly those of the hypogeous *Clathrogaster vulvarius* Petri, and those of *Russula annulata*, pseudo-angiocarpic, closely resemble the spores of

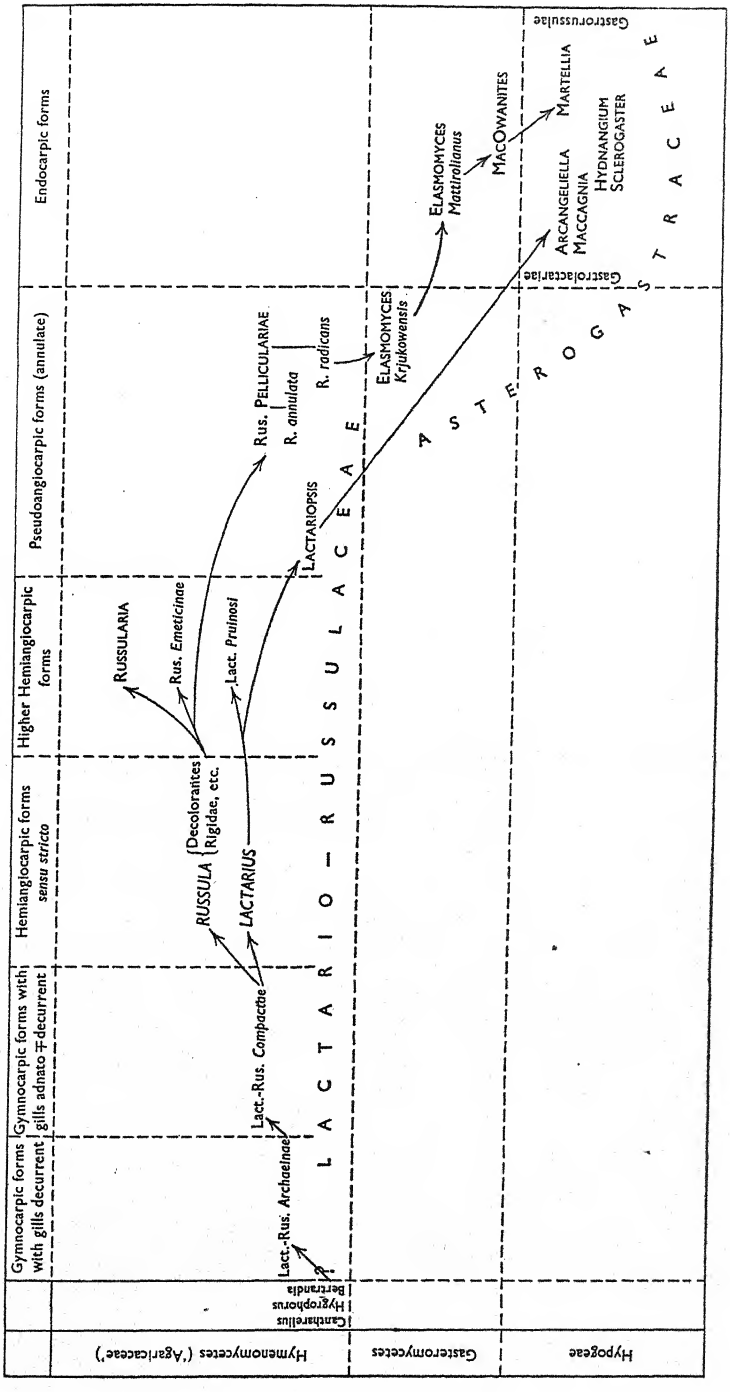
Elasmomyces russuloides Setchell; also the same fluorescent pigment, near to 'rubéine', is found in the two last species, the first one an Agaric the second a Gasteromycete. But, on the other hand, we stress the fact that the relative physiognomy of *Lactariopsis pandani* and Russulae Pellicullariae could only have arisen through convergence. We referred to *Lactarius adhaerens* Heim, with no partial veil, showing however characters of obvious inferiority (sub-lignicolous habitat, elastic consistency, spores tending to central symmetry, etc.). From this we inferred that all these forms having more or less gasteroid characters, might have resulted solely from degradations undergone in parallel directions from more evolved forms under the influence of environment. We came to the conclusion that the hypogeous or subepigeous Asterosporae 'marquaient une dégradation à partir des formes les plus proches à la fois des Lactaires et des Russules'. Soon afterwards Malençon expressed a similar opinion, saying: 'Les Asterogastraceae nous apparaissent comme une branche latérale décadente qui englobe des termes issus de *Lactarius* et de *Russula*.'

This simplification, moreover, as has been well expressed by Malençon, is reflected not only in the physiognomic characters (progressive disappearance of the stipe or of the columella, degradation of the hymenium in irregular hymenial proliferations in *Elasmomyces*, in incomplete lamelliform foldings in various Hydnangiae, etc.) but also in their anatomy: thus the basidia, nearly always tetraspored in the Lactario-Russulae, sometimes already bispored in the Pellicullariae, *Elasmomyces* and *Maccagnia*, often trispored in *Arcangeliella* and *Martellia*, tri- or bispored in *Macowanites*, bispored in *Gymnomyces* and *Clathrogaster*, are often only monospored in *Hydnangium*. We have noted that in the Asterogastraceae, even the spore also has undergone degradation, or more correctly, an abridgement or shortening of its development, and indeed does so in certain Pellicullariae where the tendency to preserve the central or axial symmetry of these elements extends even to maturity. Cystidia tend to disappear. The flesh becomes increasingly fibrous and the lactiferous hyphae less and less obvious.

These facts, taken together, uphold the hypothesis according to which the Asterogastraceae are degraded Asterosporae, adapted to a subterranean existence, while the Pellicullariae (agaricoid and russuloid) in the same way as *Lactariopsis* (lactarioid) correspond to the intermediate stages of degradation immediately preceding hypogeous adaptation, e.g. the still subepigeous *Macowanites* (Fig. 3) and *Elasmomyces* (Fig. 4), which lead in their turn to entirely closed (*Protoglossum*, Fig. 2) and subterranean forms.

Thus the series of Asterosporales, clearly distinct from all other Agaricales, includes at the same time gymnocarpic, hemiangiocarpic, pseudo-angiocarpic and endocarpic forms, namely, agaricoid, gasteroid and hypogeous, according to a phyletic continuity reconstituted on a scheme which seems satisfactory and demonstrable. Within the series of Asterosporales the fundamental distinction between Hymenomycetes and Gasteromycetes, set up by Fries and the older authors, disappears.

Table. I.


Hymenomycetes ('Agaricaceae')
Gasteromycetes
Hypogeae
Cantharellus
Hygrophorus
Bertrandia
Gymnocarpic forms with gills decurrent
Gymnocarpic forms with gills adnato ∓decurrent
Hemiangiocarpic forms sensu stricto
Higher Hemiangiocarpic forms
Pseudoangiocarpic forms (annulate)
Endocarpic forms
Gasterorussulae
Gasterolactariae
LACTARIO—RUSSULACEAE
ASTEROGASTRACEAE
Lact.-Rus. Archaeinae
Lact.-Rus. Compactae
RUSSULA
{Decoloranites
{Rigidae, etc.
LACTARIUS
RUSSULARIA
Rus. Emeticinae
Lact. Pruinosi
LACTARIOPSIS
Rus. PELLICULARIAE
R. annulata
R. radicans
ELASMOMYCES Krjukowensis
ELASMOMYCES Mattirolianus
MACOWANITES
ARCANGELIELLA
MACCAGNIA
HYDNANGIUM
SCLEROGASTER
MARTELLIA

2. Other connexions between Hymenomycetes and Gasteromycetes or Hypogeae

Other examples might be referred to, illustrating certain strong connexions between Agaricaceae and gasterobasidial forms. We shall only mention them concisely here.

(*a*) A first classical example, mentioned by Patouillard, Bucholtz and de Seynes, refers to the non-deliquescent *Montagnites* forming the bridge between *Coprinus*, a melanosporous agaric, ephemeral and deliquescent, and the gasteromycete, *Gyrophragmium* (Fig. 6), at first angiocarpic, whose hymenium shows wrinkled, contracted, labyrinthine lamellae, equivalent to a well-arranged gleba. This last genus, and also *Montagnites*, are adapted, particularly in the Mediterranean regions, to strictly arenicolous conditions and are more or less xerophytic. These form a natural melanosporous series grouping Agarics and gasteroid forms together.

(*b*) A. Maublanc and G. Malençon, in a remarkable study of the development of *Battarraea guicciardiniana* (1930*b*), pointed out the same continuity in another series: '...les genres *Tylostoma*, *Queletia* et *Battarraea* étroitement liés et formant les termes successifs d'une série très homogène. Le type le plus évolué, ou le moins dégradé, apparaît chez les *Battarraea*. Les logettes hyméniées, écho ou prélude des lames d'Agaric, y existent et la gleba contient des filaments végétatifs persistants (parois des logettes), annonçant le capillitium [qu'on trouvera dans les genres plus foncièrement angiocarpes.] Dans les *Queletia*, on n'a plus d'hyménium et le capillitium se montre nettement, quoique [d'une façon encore] rudimentaire. Les *Tylostoma* sont également sans hyménium, mais le capillitium y est très manifeste;...' By studying young forms they established that Battarraeae, previously placed among the Gasteromycetes with a homogeneous gleba, are in fact Hymenobasidiae with spore-bearing tissue arranged in small chambers in which the separating walls disappear in the fully developed fungus. Consequently the division between Plectobasidiae and Hymenobasidiae is seen to be entirely a matter of dogma.

(*c*) In 1931 (*a*), we made a study of the tropical genus *Cyttarophyllum* Heim (Fig. 8), which seems to constitute a stage between ochrosporous Agarics and Gasteromycetes of the genus *Secotium*. This fungus reminds one strongly of *Conocybe* or *Galera*, the spore being provided with a large germ-pore; it is characterized, however, by a *Podaxon* habit, namely a stipe (or columella) tightly surrounded by the margin of the pileus, so that it is almost entirely enclosed, and has a remarkably alveolated hymenium with a very slightly developed trama. The spores, with a large germ pore, and rather frequently anomalous, sometimes show two germ pores, a feature whose presence and variability recall the anomalous spores of *Montagnites* likewise occupying an intermediate position in a quite different series. In *Secotium* the hymenium shows irregular cavities, separated by anastomoses which produce the gill-like structure. *Conocybe* may lead to more evolved forms, with a very thin, ephemeral and putrescent pileus—such as in *Bolbitius*. On the other hand, *Podaxon*, with a gleba having a chambered structure and arising from wrinkled, lamellar elements, is much less clearly organized, and at

maturity, clearly plectobasidial, representing a stage still less plainly agaricoid, more plainly gasteroid, more markedly degraded than *Secotium*. In this way one can measure the range of such a series where all gasteroid as well as agaricoid elements possess certain common characteristics, such as

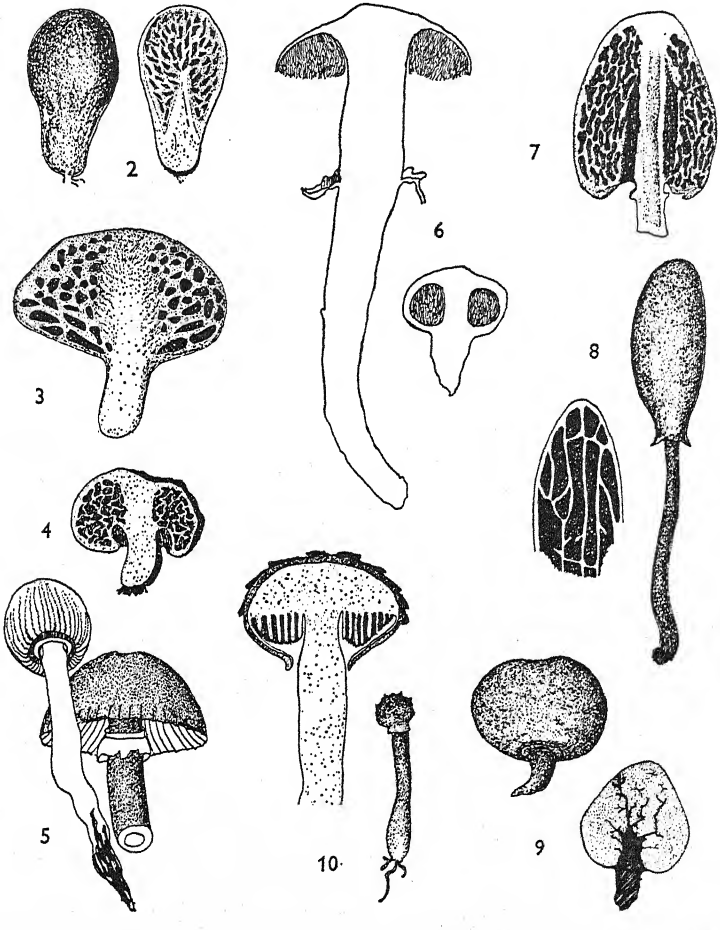


Fig. 2. *Protoglossum luteum* Massee, one specimen and its median longitudinal section (⅔ nat. size) (from Cooke).
Fig. 3. *Macowanites agaricinus* Kalch., longitudinal median section (⅔ nat. size) (from Kalchbrenner).
Fig. 4. *Elasmomyces mattirolianus* Cav., longitudinal median section (⅔ nat. size) (from Cavara).
Fig. 5. *Russula radicans* Heim, young and mature specimens (⅔ nat. size) (from Roger Heim).
Fig. 6. *Gyrophragmium delilei* Mont., longitudinal sections of immature and mature specimens (½ nat. size) (from Petri).
Fig. 7. *Secotium malinvernianum* Cesati, longitudinal median section (⅔ nat. size) (from Petri).
Fig. 8. *Cyttarophyllum besseyi* (Peck) Heim, mature specimen (⅔ nat. size) and tangential section through the pileus (½ nat. size) (from Roger Heim).
Fig. 9. *Dodgea occidentalis* Malç., one specimen and its median longitudinal section (¼ nat. size) (from G. Malençon).
Fig. 10. *Strobilomyces cinnabarinus* Heim, angiocarp, primordium and longitudinal section in a still young specimen (½ nat. size) (original drawing of Roger Heim).

ochraceous or brown spores with a large germ pore, characters which make it difficult to regard the gasteroid forms of this series as primitive, but, on the contrary, decadent.

(*d*) In 1933 our friend and colleague, Henri Romagnesi, discovered a remarkably close similarity between the spores of the hypogeous *Richoniella* and those of *Rhodophyllus*, with the same 'cristallographic' outline, and the same pink colour. 'Le manque de données actuelles concernant le *Richoniella leptoniaespora*, qui semble n'avoir été recueilli que deux fois, et dont la structure est insuffisamment connue, ne permet pas d'apporter à cette supposition une argumentation complète. Mais étant donné la position prise par Patouillard et son Ecole, relativement à la phylogénie des Basidiomycetes, nous ne pouvions laisser ignorer la valuer d'un nouvel argument aussi favorable à cette conception.' And M. Romagnesi recalls our statement of 1930, that 'les Agarics comprennent plusieurs séries parallèles indépendantes respectivement reliées à divers rameaux essentiellement angiocarpiques'.

(*e*) In 1934 we put forward the hypothesis that *Rhizopogon* was a subterranean form related to *Boletus*, a connexion already mentioned incidentally by Malençon, and we placed the tribe Rhizopogonae among the Boletaceae. Apart from having the same anatomical and spore characters, the fact that these Hypogeae may be attacked by *Hypomyces chrysospermus*, just as are certain *Boletus* spp. and *Paxillus involutus*, constituted a biological argument in favour of a relationship between the three genera: *Rhizopogon*, *Boletus*, and *Paxillus*. Malençon (1938) described a remarkable North American genus, *Dodgea* (= *Truncocolumella* Zeller, 1939) (Fig. 9), which he interpreted as intermediate between *Boletus* and *Rhizopogon*. In common with these it has a fibrous pileus, scattered and fugacious basidia, similar paraphyses, ellipsoid and smooth spores of the same colour, and shows partial gelatinization of the glebal cavity walls. But here 'l'aspect general evoque celui d'un jeune agaric dont la marge serait encore appliquée contre le stipe', because the fungus possesses a stipe narrowing towards the base, penetrating slightly into the base of the pileus, but without forming there any well-defined columella. In the Boletales series—or better in one series of Boletae—we recognize therefore three stages: (*a*) epigeous forms, gymnocarpic with tubular hymenium (*Boletus*), (*b*) hypogeous sessile forms, endocarpic with small-chambered irregular hymenium (*Rhizopogon*), and intermediately: (*c*) subhypogeous, pedicellate forms, angiocarpic and with a small-chambered, subordinated hymenium (*Dodgea*).*

The above data enabled us, in 1934, to state as our opinion: 'De plus en plus on sera conduit à admettre l'existence de séries évolutives groupant des formes angiocarpes, semiangiocarpes et gymnocarpes, à montrer combien le fossé séparant Plectobasidiés et Hyménobasidiés est fictif, à considerer les Hymenomycetes friésiens comme appartenant à des groupe-

* Similar lines uniting epigeous and underground forms have been constructed among Discales and Tuberales (cf. G. Malençon: *Les Truffes européennes*, 1938; Helen Gilkey, *Tuberales of North America*, 1938). Thus we proceed from the epigeous *Lachnea* to *Hydnocystis*, then to *Stephensia*, finally to *Tuber*, closed and hypogeous.

ments évolutifs differents, et les Agarics, notamment, comme comprenant un ensemble artificiel de formes de convergance se répartissant entre des séries phyletiques non parentes.'

3. Agarics with tubular hymenium

Thanks to material we collected in Madagascar, the Ivory Coast, and French Guinea, or received from the West Indies, or from R. Decary, we were able to study tropical Mycenaceae showing gills strongly veined or joined by transverse anastomoses, or marked simply by the exaggerated fluting of the basal hymenial plan until finally the hymenium is formed of tubes more or less independent one from the other, and thus to specify and extend the fundamental concept first realized by N. Patouillard: similarity in appearance of hymenia, contrary to the strictly morphological viewpoint of El. Fries, does not constitute an absolute criterion of relationship among the Hymenomycetes; the same natural series show side by side genera whose hymenial surfaces have a very different aspect.

Such fungi might be placed in *Mycena*, properly so-called, as *M. longicystis* Heim from Guadeloupe; *Hemimycena* whose dense but shallow, transverse folds form a supernumerary hymenial net; a group which may be named *Dictyoploca* Mont., assembles *Collybia*-like forms showing subdecurrent gills whose numerous veins form true alveoles measuring from 700 μ to 4 mm. diameter according to the species (*D. myrmecophila* Heim, Fig. 14; *D. heterophylla* Heim, Fig. 11); the ochrosporous genus *Phaeomycena* Heim from Madagascar shows a hymenium regularly reticulated between the radial lamellae (Fig. 13); in the genus *Phlebomycena* Heim, also from Madagascar, appears a complete structure of lateral anastomoses leading to the formation of regular hymenial cups from 600 to 1200 μ diameter, occupying the whole hymenial surface (*P. medecassensis* Heim, Fig. 15).

Poromycena from Java, described by van Overeem, and since found again in Madagascar, sometimes with what look like real specific mutations, appears to be derived from lamellated *Mycenas*, *Poromycena decipiens* v. Over. (Fig. 12) differing from *Mycena pseudopura* Cke. only in having the hymenium formed of alveoles of 500–900 μ diameter: habit, colour, taste, smell, anatomical and spore characters agree. However, our observations show that the reticulated appearance of the hymenium has no specific stability, even on carpophores arising from the same mycelium. The distinction between *Poromycena decipiens* and *Mycena pseudopura* is based only on a variable quantitative character. On the contrary, *Poromycena manipularis* (Bk.) (Fig. 16) shows a hymenium perfectly and consistently tubular, including spores which become grey (just as the subcuticular layer becomes red) by the action of iodine, pores of 0·5 mm. diameter, scarcely orientated in a radial direction, with a slight dominance of the true lamellar planes.

The South American *Laschia clypeata* Pat. (*Mycenoporella*) (Fig. 17), which we also have found on the Ivory Coast, represents a mycenoidal type with a perfect tubular hymenium, with tubes measuring 130 μ diameter, without any indication of radial arrangement and the spores and hypophyll are amyloid.

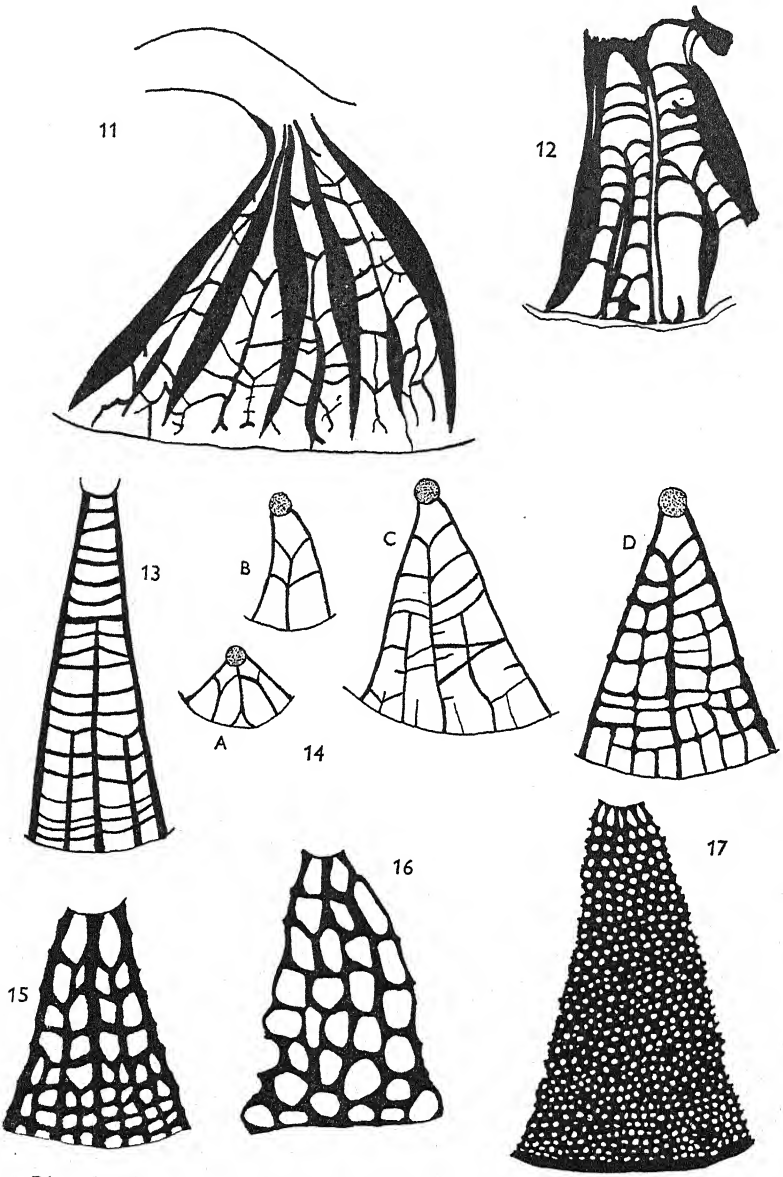
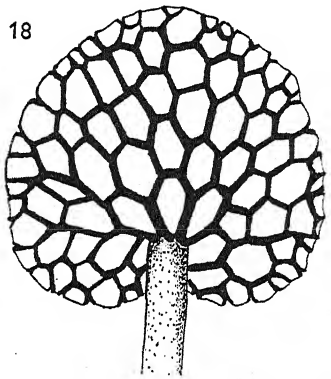


Fig. 11. *Dictyoploca heterophylla* Heim, from Guadeloupe, aspect of a part of the hymenium in schematized profile (×4).
Fig. 12. *Poromycena decipiens* van Over., from Insulinde and Madagascar, as above (×8).
Fig. 13. *Phaeomycena aureophylla* Heim, from Madagascar, hymenial plane between two principal lamellae (×4).
Fig. 14. *Dictyoploca myrmecophila* Heim, from Madagascar, four successive evolutionary stages, in a plane of a hymenial sector between two principal lamellae (×4).
Fig. 15. *Phlebomycena madecassensis* Heim, from Madagascar, hymenial sector in one plane (×4).
Fig. 16. *Poromycena manipularis* (Berk.), from West Africa and Madagascar, as above (×4).
Fig. 17. *Mycenoporella clypeata* (Pat.), from South America and West Africa, as above (×4).

These various fungi, connected by numerous common characters, join on to the Agaricaceae at a level with *Collybia*, *Mycena*, *Omphalia* and also *Marasmius*. The assembling of the material concerned led us to note a progressive continuity in the formation of the net and the construction of the tube, starting from true *Mycena* with simply veined lamellae up to *Mycenoporella clypeata* with a hymenium showing identical and equidistant small pores, representing an end-term of development in the agaricoidal tubular hymenium. The dynamic value of the anastomosing vein shows itself in an exaggerated manner in intraspecific mutation (*M. pseudopura*).

All these species have a slender habit and an exactly central stipe. But another series of Agaricaceae, heterogeneous, though related to *Mycena*, comprising the major portion of *Porolaschia* and also *Favolaschia* (the latter with a pellicle composed of brushy cells), unites pleurotoid forms, either pleuropodial or apodial, with reticulated, often meruloid or cantharelloid, hymenia (Fig. 18). The consistency here becomes gelatinous. Alveoles may reach large dimensions in a regularly hexagonal form: a tendency to



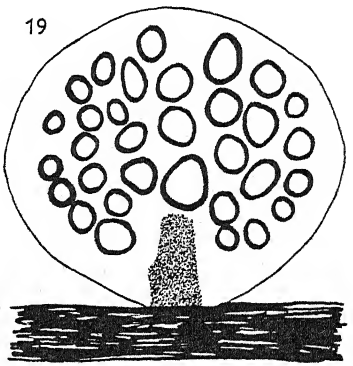


Fig. 18. *Favolaschia calocera* Heim, from Madagascar, view in the hymenial plane (×3).
Fig. 19. *Mycomedusa guineensis* Heim, view in the hymenial plane of a young specimen.

a resupinate habit is obvious. In short, the similarity with Polypori and Favoli increases. The gelatinization of the receptacle leads to the genus *Mycomedusa* Heim (Ivory Coast), with completely gelatinous flesh except around some large cavities on the lower surface bordered by a hymenial palisade of basidial cells. Here the carpophore has undergone an extreme simplification linked with a biochemical factor.

To conclude, just as one knows Boleti with lamellated (*Paxillus*) or alveolated hymenium (*Phylloporus*), there are different groups of agarics with tubular hymenia, most of them characteristic of *Mycena* (incl. *Collybia*), consequently without any relationship either with Polypori or Boleti. The two series which group together such fungi, seem conditioned by adaptation to habitat and by consistency respectively, on the one side a tendency to an entirely lignicolous existence—a pleuropodial then resupinate stage, the acquirement of a gelatinous consistency, paucity of tubes, generally polygonal in section (*Favolaschia*)—on the other hand, preservation of the axial

Table 2. *Recapitulation table of the principal species of mesopodal Agarics with tubulated hymenium (according to Roger Heim, 1945)*

Species	Taste	Spores	Iodine reaction	Cystidia, *C*, or cystidial hairs *PC*	Average diameter of hymenial alveolae	Mode of hymenial anastomosis
Mycena decaryi	—	9–10 (–11) × 5·5–6·5 μ	+	o	—	Very tightly folded transverse anastomoses
M. longicystis	Rather peppery	12·5–15·5 × 4·5 μ	–	*C*	—	Numerous anastomosing veins
Dictyoploca myrmecophila	Turnip	7·5–9 × 3·5–4·5 μ	–	*PC*	3000–4000 μ	Numerous regular anastomoses, heterogeneous pores
D. heterophylla	?	6–6·9 × 4–4·8 μ	–	*PC*	1000–2000 μ	Folds and anastomoses, heterogeneous and irregular pores
D. guadalupensis	?	5·7–6·8 × 3·6–3·8 μ	–	*PC*	700–1000 μ	Transversal basal folds, very heterogeneous pores
Phaeomycena aureophylla	Rather bitter	6–8 × 4·5–7 μ	–	o	± 1000–4000 × 1000 μ	Irregular and close transverse folds; elongated, concentric pores
Phlebomycena madecassensis	Radish	7–8·6 × 6–6·4 μ	+	*PC*	600–1200 μ	Regular and entire alveolated hymenium, but lamellar planes predominant
Poromycena decipiens	Black radish	6–7 × 4–5 μ	±	*PC*	500–900 μ	Network becoming more obvious
P. brunnea	?	7–8 × 3–2 μ	?	o	500–900 μ	,, ,,
P. manipularis	Turnip	6·6–7·6 × 4·5–5·5 μ	+	*PC*	600–700 μ	Network nearly perfect and entire
Mycenoporella lutea	Unobtrusive	5–6·6 × 3·5–4·5 μ	?	o	100–250 μ	Hymenium perfectly tubular
M. clypeata	Not noted	6–7·2 × 5·2–6·5 μ	+	*PC*	130 μ	Hymenium perfectly and entirely tubular

symmetry and the epigeous life by a slender habit and thin flesh, acquirement of a more tenacious consistency, and a multiplication of the tubes, which are round or quadrangular in section (Mycenae, Collybiae).

Thus we find in both series various successive stages of transformation from a lamellated to an alveolated hymenium, the two extreme types being characterized in one as in the other section by the same poroid or tubular stage attaining an equal degree of development. *Mycenoporella clypeata* among the mesopodial species; *Mycomedusa guineensis* (Fig. 19) among pleuropodial species. It is impossible in one as in the other of the two series to fix a systematic limit between lamellated and tubular forms: the importance of the Friesian distinction, which opposed these two hymenial arrangements, disappears.

4. Heterogeneity of the Polyporeae in the Friesian system

Just as the Friesian notion of the Agaricinae excluded from this family all Hymenomycetes which did not possess a lamellated hymenium, that of Polyporaceae brought together all those having a tubular hymenium, and only those. N. Patouillard in his first writings, and especially in his 'Essai Taxonomique' formulated with clarity, crucial criticisms against this point of view. He wrote: 'La surface hyménifère peut revêtir des aspects differents dans des genres voisins et inséparables. Les caractères tirés de cette ornementation ne pourront servir à caractériser les familles, mais seulement à délimiter les genres. Dans chaque famille, les genres sont extrêmement voisins et ne montrent nettement leurs caractères distinctifs que dans leurs formes centrales.' First he separates the Boleti from the Polypori, associating *Boletus* with the lamellated agarics. He includes the Boleti in the large family Agaricaceae, dividing them into two groups, one, *Boletus*, with a tubular hymenium, the other, *Paxillus*, with a lamellate hymenium. *Phylloporus*, with a hymenial surface which is both lamellated and alveolated, forms a connecting link.

In course of time this view has been essentially confirmed. The new relationship between *Boletus* (*Xerocomi*) and *Paxillus*, with the intermediate genus, *Phylloporus*, bearing lamellae strongly anastomosed behind or over their whole length, has been supported by other criteria: the easily separable hymenium—whether it be poroid, lamellated or alveolated—its flaccidity, the tendency to shrivelling, also the anastomoses between the lamellae, especially in the neighbourhood of the insertion on the stipe. Josserand has shown (1932) that the trama in the lamella of *Paxillus* is similarly bilateral, although less clearly so than in *Phylloporus*. The spore characters are similar. Finally, the pigmentation in the three genera *Paxillus*, *Phylloporus* and *Xerocomus* is very close. So from species to species we get the links of an unbroken chain proceeding at least from *Paxillus panuoides* with agaricoid lamellae, practically unforked, up to *Xerocomus* of the group *subtomentosus*, entirely boletoid.

With regard to the separation of the genuine Boleti and the Polypori, this seems on the whole to be firmly established. The indefinite mode of growth of the hymenium of Aphyllophorales, which includes lower forms

showing a smooth hymenium and forms with hydnate and pored rugulose hymenia, characterizes an assemblage of species with a tenacious, for the most part non-putrescent flesh, generally lignicolous, never mycorrhizal, while nearly all the genuine Boleti are non-lignicolous and probably all mycorrhizal, with a putrescent flesh, which is seldom tenacious.

However, links between '*Boletus*' and '*Polyporus*' seem to occur in the three genera: *Gyrodon*, boreal (as already mentioned by Singer); *Phlebopus* Heim, African-Madagascan; and the Madagascan *Ixechinus* Heim, peculiar to the chlaenaceous forests. These two last genera show characters which bring them close to the Polypori: *Phlebopus* by the false sclerotium, the excentric stipe and narrow hymenium; *Ixechinus* by the tough stipe and the more or less lignicolous habit. Moreover, the tubes, in this last genus, are free one from the other; the tube separates from its neighbours through the gelatinization of the trama between the primitive tubes, and consequent rupture. This mechanism of chemical and mechanical disjunction of the hymenial elements removes *Ixechinus* from the Fistulinae, whose stuffed tubes, discoid in reality although entirely free from one another, have a very different origin: they are connected with *Cyphella* and *Solenia*. To Lohwag and Follner's (1936) arguments we add those of a chemical nature: Fistulinae are the only fungi known to contain d. arabitol and the only ones—or almost the only ones—in this Polyporae group in the Friesian sense which do not contain mannitol; finally they possess a latex of a very special nature. These tubular fungi are quite distinct from the Boleti and from the genuine Polypori.

Lastly, like E. J. Gilbert (1931), we separated *Strobilomyces* from the Boleti: the species of this genus show a light structure, complex and ornamented spores, are not mycorrhizal, but often lignicolous and differ from the genuine Boleti—which contain boletol and no strobilomycol—in containing strobilomycol and boletol, according to Gab. Bertrand. In a Madagascan *Strobilomyces* with a persistent annulus (Fig. 10) we have been able to demonstrate the *angiocarpic* nature of the hymenium—the primitive origin of the annulus in contrast with its mode of development in *Boletus*, which is *pseudo-angiocarpic* as shown by Kühner (1926). Thus *Strobilomyces* is to be considered as a gasteroid form like *Elasmomyces* or *Gyrophragmium*.

But if these different sections must be removed from the sphere of the Polyporae or Boletae, if the gap between Boletales and Aphyllophorales is truly profound, it seems justifiable on the other hand to bring several lamellated groups nearer to the Polypores, placed up till now among the Agarics because of their lamellated hymenium. If the lamellated Paxilli are Boleti and if Mycenoporellae are Agarics, then in the same way *Lentinus*, in spite of its lamellae, and *Schizophyllum*, also lamellated, are most certainly better placed among the Polypori.

On the contrary, species with a tubular hymenium and tenacious flesh, like *Polyporus scobinaceus*, are very close to *Calodon*, i.e. it represents a *Polyporus* with hydnated hymenium. This last remark, conforming with the view of Donk and Singer, ought not to be surprising when one considers the capacity for variability of the hymenium in certain species of Aphyllophorales. The following instances are classical: *Lenzites tricolor*, lamellated,

is specifically inseparable from *Trametes rubescens*, tubular; *Irpex fuscoviolaceus*, hydnated, from *Coriolus abietinus*, tubular; *C. versicolor*, in culture, may show a hydnated hymenium whose spines are joined secondarily into tubes, etc. We do not enlarge further upon these remarks, for they are already well known.

These instances show, therefore, that the term *Boletus* has lost its meaning: certain Boleti, in the Friesian sense, are tubular Agarics near to *Mycena*, certain Agarics are lamellated Boleti near to *Xerocomus*, certain Boleti are assimilable to Gasteromycetes (primitively angiocarpic), Polypori may possess a lamellated hymenium. Agarics, *Boletus*, *Polyporus*, Gasteromycetes, are four terms which have lost their original, too exclusively physiognomical significance.

5. Influence of cytological data

Logically the foregoing facts lead us to conclude first that we cannot have *one* group of Agaricaceae and *one* group of Gasteromycetes, and secondly that in each natural series connecting agaricoid and gasteroid forms, these last are to be interpreted as evolved forms subsequently degraded and not as the most primitive.

In fact, the subject is a thesis upon which authors, even ancient authors, have expressed their opinion, but we think that in general they have misinterpreted not the evolutionary succession, but its direction.

Thus H. Lohwag has also realized that a close connexion exists between the Agaricinae and certain Gasteromycetes. However, his confused views brought together probable and imaginary connexions inspired by the ideas of Brefeld, and von Höhnel. Starting from his anxiety to discover organic homologies, he interpreted the genera *Amanita*, *Lepiota*, *Armillaria* and *Volvaria*, as phylogenetically more primitive than the forms without annulus and volva. He regards these two last structures as superfluous, being maintained only in a minority of Agaricaceae. Thus he is led to the opinion that the latter are derived from the Secotiaceae. Singer (1936) goes much further: he considers that there is only one line of descent of Agaricales from Gasteromycetes, basing his hypothesis notably on the fact that in Russula 'les formes à voile sont précisément primitives'. But this last assertion is wholly gratuitous and we have endeavoured to explain why it is more likely that they are simply degraded. Moreover, to support one's views on a very uncertain reconstruction of the tendencies within a genus and then to deduce from it theoretical ideas explaining the general evolution of an assemblage of genera, appears to us to be very much open to dispute. In fact, Lohwag and Singer did not understand how the veil could *acquire* an hereditarily protective value. Without developing this point we refer to our memoir on *Termitomyces* (1941) where the adaptative value of veils (general, partial and marginal) seems to be demonstrated.

But the exact support of cytological data comes to the aid of anatomy and morphology. In a recent and very intersting treatment based upon numerous cytological observations, Kühner (1945) arrives at conclusions perfectly compatible with ours. For him also 'les voiles se rencontrent surtout chez les espèces relativement différenciées par ailleurs'.

He remarks that the gymnocarp types of Agaricales are found chiefly among forms 'à paroi sporique mince et à stipe confluent avec le chapeau' and never among those where the spore possesses a germ pore and a high differentiation 'à une foule de points de vue'. He concludes that 'Si l'on admet que les espèces les plus différenciées sont les plus évoluées, on se trouve donc conduit à l'hypothèse soutenue par Fayod, selon laquelle les Agaricales primitives étaient dépourvues de voile', a hypothesis diametrically opposed to that of Brefeld and of Singer.

Kühner considers that the presence of clamp connexions is a primitive character, and he supports his opinion with strong evidence. But, Amanitae and Lactario-Russulae are rich in species without clamp connexions, while *Cantharellus* and *Mycena* mostly have them, at least in non-parthenogenetic forms. In like manner *Psalliota* is always without clamp connexions and *Tricholoma* often so.

Moreover, in the relatively differentiated species, within quite natural genera, one finds multinucleate segments, while more simple forms show a very reduced number of nuclei. Better still a correlation between the number of nuclei in the spore and the degree of differentiation seems well founded: the most evolved genera with white spores, like *Amanita*, show *two* nuclei in the spore, while more primitive genera like *Cantharellus*, show only *one*. In *Tricholoma*, species with one nucleus prevail. Tribes placed below *Tricholoma* by Fayod, seem to include only types with uninucleate spores.

Kühner concludes that 'ces arguments d'ordre cytologique appuient l'hypothèse selon laquelle l'évolution des Agaricales aurait été progressive dans ses grandes lignes' from primitive, less differentiated types, among which gymnocarpic types prevail, towards the most differentiated types generally provided with a veil.

This is an important cytological confirmation of the thesis we are upholding.

But there is also another. It concerns the discovery, in the Phalloidae, in large polyhedral cells of the stipe recalling those of the parenchyma in the higher plants, of chromoplasts containing carotene crystals, absolutely similar to those of *Clivia* and *Gladiolus*. This important discovery, published quite recently by Mme Panca Heim (1946), brings fresh support to our thesis that the Phalloidae, Gasteromycetes adapted to hygrophilous conditions of life, *constitute quite the most evolved group*, the end group of Basidiomycetes, and also that the Gasteromycetes with a pulverulent gleba represent forms adapted more generally to xerophytic or mesophytic conditions of life, *showing a morphological and convergent degradation and simplification which only marks their evolved stage within a distinct natural series*.

The hypothesis of our friend R. Singer seems to be refuted by these new arguments also.

But it remains to specify the definition of the natural groups whose limits have been overthrown by recent works. If the Boleti and Polypori are no longer characterized by tubes, Hydnae by spines, Agarics by gills, and Gasteromycetes by an endocarpic structure, how shall we find our way about among the intricacy of forms classified by Fries, undoubtedly

artificially, but with a practical result that has proved very advantageous? Will it be necessary to refer to an assemblage of characters of which the utilization will be difficult and not absolute? We think that the large groups are sufficiently clarified by some characters to which, however, a new importance must be given: relations obligatorily positive or negative in respect to the vegetation, nutritional features, particulars of symmetry or asymmetry in the definite or indefinite growth of the hymenium and the carpophore, consistency, certain biochemical characters, etc.

We have tried, in these few pages, to summarize briefly recent studies published chiefly by French mycologists, on the natural position of the principal groups of Macromycetes, based on anatomical, embryological and biochemical researches. From these it follows that only from a synthesis of the data resulting from the use of various methods will it be possible to establish a natural classification of fungi, and finally to map out their phylogeny on a solid basis, no longer inspired merely by similarities, resemblances and physiognomical convergence.

REFERENCES

Bucholtz, F. (1902*a*). *Beiträge zur Morphologie und Systematik der Hypogaeen.* Riga.

Bucholtz, F. (1902*b*). Zur Morphologie und Systematik der Fungi Hypogaei. *Ann. Mycol. Berl.* I, 152–74.

Cavara, F. (1897). Contributo alla conoscenza della Podaxinae (*Elasmomyces mattirolianus* nov.gen. et sp.). *Malpighia*, XI, 414–28.

Cooke, M. C. (1892). *Handbook of Australian Fungi.* London.

Fayod, M.-V. (1889). Prodrome d'une histoire naturelle des Agaricinés. *Ann. Sci. nat.* sér. VII, Bot., IX, Paris.

Gilbert, E. J. (1931). *Les Bolets.* Paris.

Heim, Mme. Panca (1946). Sur la localisation des pigments carotiniens chez les Phalloïdées. *C.R. Acad. Sci.* CCXXII, 1354–5.

Heim, Roger (1931*a*). Sur les liens phylétiques entre les Agarics ochrosporés et certains Gastéromycètes. *C.R. Acad. Sci.* CXCII, 291–3.

Heim, Roger (1931*b*). Le genre *Inocybe*, précédé d'une Introduction générale a l'étude des Agarics ochrosporés. *Encycl. mycol.* I, P. Lechevalier ed., Paris.

Heim, Roger (1934). *Fungi iberici. I. Observations sur la flore mycologique catalane.* Barcelone.

Heim, Roger (1935). Spécificité et filiation chez les Hyménomycètes. *Proc. Zesde Intern. Bot. Congres*, II, 150–2, Amsterdam.

Heim, Roger (1936*a*). Sur la parenté entre Lactaires et certains Gastéromycètes. *C.R. Acad. Sci.* CCII, 2101–3.

Heim, Roger (1936*b*). Sur la phylogénie des Lactario-Russulés. *C.R. Acad. Sci.* CCIII, 108–10.

Heim, Roger (1936*c*). Observations sur la flore mycologique malgache. III. Trois Bolets gigantesques d'Afrique et de Madagascar. *Rev. mycol.* I, 3–17.

Heim, Roger (1937). Observations sur la flore mycologique malgache. V. Les Lactario-Russulés à anneau: ontogénie et phylogénie. *Rev. mycol.* II, fasc. 1, 2, 3.

Heim, Roger (1938). *Les Lactario-Russulés du domaine oriental de Madagascar. Essai sur la classification et la phylogénie des Astérosporales.* Paris.

Heim, Roger (1939*a*). Sur l'existence de Bolets à tubes libres. *C.R. Acad. Sci.* CCVIII, 375–7.

Heim, Roger (1939*b*). Observations sur la flore mycologique malgache. VII. Les Bolets à tubes libres. *Rev. mycol.* IV, 5–20, 6 figs., 1 pl.

Heim, Roger (1940). A propos des Russules tropicales à voile membraneux. *C.R. Acad. Sci.* CCXI, 74–6.

HEIM ROGER (1941). Études descriptives et éxperimentales sur les Agarics termitophiles d'Afrique tropicales. *Mém. Acad. Sci. Inst. France*, LXIV, 1–74.

HEIM, ROGER (1942). Les pigments des champignons dans leurs rapports avec la Systématique. *Bull. Soc. Chimie Biol.* XXIV, 48–79.

HEIM, ROGER (1943*a*). Remarques sur les formes primitives ou dégradées de Lactario-Russulés tropicaux. *Boissiera*, VII, 266–80, 3 figs.

HEIM, ROGER (1943*b*). Sur l'existence d'Agarics tropicaux a hyménium tubulé. *C.R. Acad. Sci.* CCXVII, 157–9.

HEIM, ROGER (1945). Les Agarics tropicaux à hyménium tubulé. *Rev mycol.* X, 3–61.

JOSSERAND, M. (1932). Sur la nature de la trame dans les genres '*Paxillus*' et '*Phylloporus*'. *Bull. Soc. mycol.* Fr. XLVIII, 112–13.

KALCHBRENNER, C. (1876). Zwei neue Pilzgattungen. *Hedwigia*, XV, 115.

KÜHNER, R. (1926). Contribution à l'étude des Hyménomycètes et spécialement des Agaricacés. *Botaniste*, sér. XVII, fasc. I–VI, 2–224.

KÜHNER, R. (1945). Le problème de la filiation des Agaricales à la lumière de nouvelles observations d'ordre cytologique sur les Agaricales leucosporées. *Bull. Soc. linn. Lyon*, pp. 162–9.

LOHWAG, H. (1924*a*). Zur Stellung und Systematik der Gastromyceten. *Verh. zool.-bot. Ges. Wien*, LXXIV, 38–55.

LOHWAG, H. (1924*b*). Entwicklungsgeschichte und systematische Stellung von *Secotium agaricoides* (Czern.) Holl. *Öst. bot. Z.* pp. 161–74.

LOHWAG, H. & FOLLNER, L. (1936). Die Hymenophore von *Fistulina hepatica*. *Ann. mycol. Berl.* XXXIV, 456–64.

MAIRE, RENÉ (1902). Recherches cytologiques et taxonomiques sur les Basidiomycètes. *Bull. Soc. mycol. Fr.* XVIII, 210 pp.

MALENÇON, G. (1931). La Série des Asterosporés. *Trav. cryptog. dédiés à Louis Mangin*, pp. 377–96, Paris.

MALENÇON, G. (1935*a*). Etudes sur les Phellorinés. I. Le *Phellorinia Delestrei* (Dur. et Mtgn.). Ed. Fischer. *Ann. Crypt. exot.* VIII, 5–48.

MALENÇON, G. (1935*b*). Etudes sur les Phellorinés. II. Le *Dictyocephalus curvatus* Underwood. *Ann. Crypt. exot.* VIII, 101–33.

MALENÇON, G. (1938). *Dodgea occidentalis* Malençon, nouveau genre et nouvelle espece de Rhizopogoneae. *Bull. Soc. mycol. Fr.* LIV, 193–203.

MALENÇON, G. (1942). La sporogénèse chez les Basidiomycètes. *C.R. Acad. Sci.* CCXV, 484–6.

MATTIROLO, O. (1924). Funghi africani raccolti dal Dott. Giovanni Negri nella Etiopia meridionale. *Atti Accad. Torino*, LIX.

MAUBLANC, A. et MALENÇON, G. (1930*a*). Sur la nature et l'organisation de la gleba du *Battarraea Guicciardiniana* Ces. *C.R. Acad. Sci.* CXC, 510–12.

MAUBLANC, A. et MALENÇON, G. (1930*b*). Recherches sur le *Battarraea Guicciardiniana* Ces. *Bull. Soc. mycol. Fr.* XLVI, 43–73.

PATOUILLARD, N. (1887). *Les Hyménomycètes d'Europe*. Paris.

PATOUILLARD, N. (1890). Sur la place du genre *Favolus* dans la classification. *Bull. Soc. mycol. Fr.* VI, 19–21.

PATOUILLARD, N. (1898). Note sur une déformation polyporoïde du champignon de couche. *Bull. Soc. mycol. Fr.* XIV, 46–7.

PATOUILLARD, N. (1900). *Essai taxonomique*. Lons-le-Saulnier.

PETRI, L. (1909). *Gasterales. Flora italica cryptogama*. Florence.

RAMSBOTTOM, J. (1940). Taxonomic problems in fungi. In *The New Systematics*, pp. 411–34, ed. J. Huxley.

ROMAGNESI, H. (1933). Le genre *Richoniella*, chaînon angiocarpe de la série des Rhodogoniosporés. *Bull. Soc. mycol. Fr.* XLIX, 433–4.

SINGER, R. (1936). Das System der Agaricales. *Ann. mycol. Berl.* XXXIV, 286–378.

SINGER, R. (1939). О новых данных для систематики и филогении Agaricales как потомков Gastromycetes. „Советская ботаника", no. 1, 95–8.

SINGER, R. (1945). The *Laschia*-complex (Basidiomycetes). *Lloydia*, VIII, 170–230.

CLOSING REMARKS

By THE PRESIDENT

THE PRESIDENT, in closing the session, said that they had no reason to doubt that the Anniversary celebrations had succeeded even beyond the great expectations some had harboured. The scientific contributions had been of the highest standard, and where there had been opportunity for discussion this had been of the free, friendly and open kind that augured well for mycology, and for that essential international understanding among mycologists. The animated conversations both general and amongst our guests, the happy groups of those interested in special problems, the small convivial gatherings, all showed how, if left to themselves, mycologists could gladly tackle their own problems in friendly co-operation.

He had been deeply moved during the brilliant exposition they had just heard delivered with typical French *élan*, by the thought that eighteen months ago Prof. Heim was still resisting final efforts to exterminate him at Mauthausen. It seemed symbolic of their high hopes that so soon after the cessation of world-wide hostilities men of many nations should listen to such a discourse with enthusiasm and without political rancour. The whole atmosphere was different from that of 1944 when, in a period of blitz and black-out, the audience had not dallied after a course of lectures he had himself given in that splendid lecture theatre. No such scurry was to be anticipated to-day, for all were only too well aware that the formal proceedings were over and that there was little time left for all that remained to be said. He thanked all those who had contributed to the programme or helped with the arrangements, and particularly our foreign guests who by their presence had assured the success of the Jubilee Meeting, and had made easier the approach to international co-operation in the furtherance of the study of mycology in all its branches. On behalf of the members of the British Mycological Society he wished them *bon voyage*.

CARLETON REA

By J. RAMSBOTTOM

Carleton Rea died at his home in Worcester during the night of 26 June 1946. To older members of our Society he *was* the Society, for it was mainly due to his energies that it was founded and continued. He was the first Secretary and Editor, and served as Treasurer after the first year. He carried on in these offices until 1918, when being appointed General Secretary he relinquished the first and last, but remained as one of the Editors until 1930. He was President in 1907, and again in 1921.

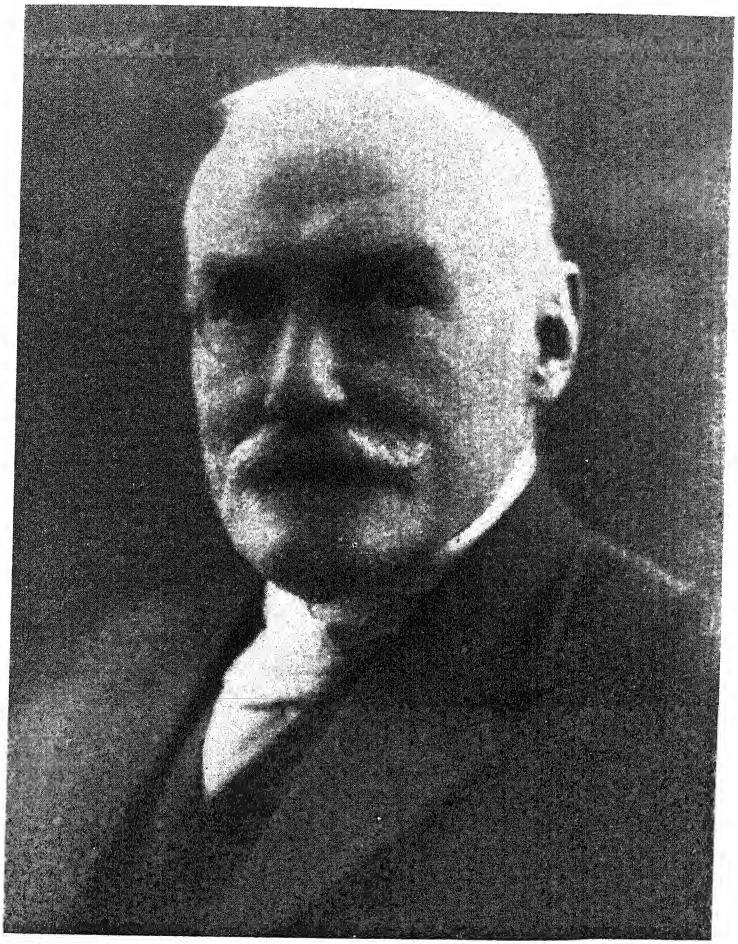
He was born in Worcester on 7 May 1861, and came of an old Worcester family. He was the only son of R. Tomkins Rea, formerly City Coroner and Clerk of the Peace.

He was educated at King's School, Worcester, and later at Magdalen College, Oxford, where he studied law and obtained his B.C.L. He entered the Inner Temple and when called to the Bar joined the Oxford Circuit. Though proud of his profession he did not apply himself with the energy necessary for success, and from about 1904 took fewer cases and finally ceased practice in 1907. He told me that he retired as he found that his earnings merely covered his expenses. As he did not scorn delights his days were not laborious: billiards seem to have figured more in his activities than did cases. Truth to tell neither his manner nor his speech were such as to inspire the confidence necessary for legal success. I was surprised when I first heard him speak at our meetings, for he was difficult to follow. In later years he seemed to improve in his manner of address: he was certainly less blustering though never less *fortiter in re*. His reading of a paper was nearly always an affliction on the audience, for he read badly and would delight in making the subject-matter mostly strings of names.

It always puzzled me how one so apparently careless of success in a profession where great plums are to be gained by application and a critical faculty, should have laboured so assiduously and have shown those very talents in mycology. During the 1914–18 war he did invaluable work on a Tribunal of the Ministry of National Service—for the rest he was active in his leisure.

As a schoolboy he became an ardent naturalist. He joined the Worcestershire Naturalists' Club at the age of fifteen and later was for many years its Secretary, and was frequently its President as he was in 1946, its centenary year. He edited the volume of the first fifty years of its *Transactions*, and continued as Editor, thus as he proudly said, he had covered the hundred years of the Club's existence. He was regular in his attendance on field days, more often than not acting as leader: his last excursion was only a few days before his death.

He appears to have included fungi in his boyhood interests, possibly because of the fame of the Forays of the neighbouring Woolhope Club, but it may have been that the Worcestershire Naturalists still felt the influence



CARLETON REA

of its first President, Edwin Lees, who is known to mycologists chiefly through his theory of the formation of fairy rings by the action of moles, and who had led an autumn excursion (Fungus Foray) in the Club's first year.

He was a keen entomologist and assiduously collected insects of all kinds. He was also a critical field botanist and collaborated with J. Amphlet in the *Botany of Worcestershire*, which was published in 1909 and which he kept up to date with several supplements. He had a wide knowledge of garden plants.

He was a naturalist of the old school, delighting in everything to be met with in field and woodland. His specializing in 'fungus' as he always called them, seems to have been due to his friendship with Amy Rose, who in 1898 became Mrs Rea. She was a member of the Worcestershire Naturalists, as also was her father, John Rose, a prominent Worcester solicitor, who later attended the Mycological Meetings of the Yorkshire Naturalists, and was one of our Society's original members. She wanted her specimens to be named and Rea agreed to do this if she would paint his fungi. The result was the beginning of the large series of paintings which are eventually to go to the Department of Botany, British Museum.

Drawings or specimens were occasionally sent to M. C. Cooke, and later, to G. Massee. Cooke certainly encouraged Miss Rose in her efforts and he gave her friendly criticism as well as high praise: the best painting I know by Cooke was sent to her as a Christmas card and always hung in Rea's study—it showed a toad sheltering under *Cortinarius caerulascens* amongst beech leaves.

Rea's early interest in mycology is seen in the account of a meeting of the Club in October, 1892, which it is said 'will be ever memorable in the annals of the club by reason of the fact that two eminent specialists, Dr M. C. Cooke, M.A., A.L.S. and Mr George Massee, F.R.M.S., F.L.S., the highest authorities in mycology, had, through the instrumentality of our member Mr C. Rea, been prevailed upon to accept the invitation of the club to attend their fungus foray'.

Rea's first paper was 'Notes on Plants of Rare Occurrence in the Severn Valley', read before the Worcestershire Naturalists in 1892; his next, two years later, were 'Notes on Birtsmorton Court' and 'Notes on Painswick Beacon'. Meanwhile, an indication of specialization is a note in the last number of *Grevillea* (XXII (1892), 121): 'Mr C. Rea who is desirous of making an exhaustive examination of the Russulae, would be greatly obliged if British and Continental mycologists would kindly aid him in the study by forwarding fresh specimens to 34 Foregate Street, Worcester, during the present and succeeding season.' *Russula* was always a favourite genus with him, but later was second to *Cortinarius*. In 1895 he described *Russula luteotacta* (*Trans. Worc. Nat. Cl.* 1847–96, 416 (1897)).

I first met Carleton Rea at the Taunton Foray in 1911. Miss Lorrain Smith had often spoken about him to me in the previous months, so I was not altogether unprepared. He was standing in a group in the Castle grounds and was dressed in the style he affected to the end, and which had probably been little modified since his young days, for he was very conserva-

tive in dress as he was in politics. Early photographs show him wearing a brown bowler, but it was almost always a panama in my time, with a trilby occasionally: always knickerbockers—he was an ardent cyclist until quite late in life—except when visiting London or when dining: always a high single collar, white shirt with prominent cuffs and gold links; a white tie and usually a white waistcoat; a monocle usually, without any attachment, but occasionally spectacles in the field. He was ruddy of countenance and always used a holder when smoking cigarettes, and smoked cigarette tobacco in his pipe. He liked to have a walking stick on excursions, but he would have scorned to have used it for propulsion; it was for beating down brambles or bracken or for aid in scrambling down slippery slopes. His handwriting was very small and clear, surprisingly so considering his very shaky hand; he used a very small writing paper and envelope, and always a penny pocket note-book for making his Foray lists. He was a period piece of the naughty nineties, but always a boy at heart.

My long and valued friendship with him dated from our first meeting. I was practically ignorant of field mycology—to be honest, ignorant also of many other branches of the subject—but as I didn't pretend, he was delighted to encourage, or as I found out later, proud to do so, and, moreover, had decided that I should be useful to the Society.

As I could not attend the Spring Foray at Worcester in 1912, he invited me to spend the previous week-end with him at the Queen Anne House, 34 Foregate Street, where he had been born and where he resided until 1918. He met me at the station as he always did later, and we drove in a four-wheeler. My visit lasted over three nights and on none of them did we retire before 3 a.m. These talks after long days in the field were always interesting and informative, but were not so prolonged after some years, for one o'clock was the signal then for beginning to move. My recollection of the first morning delights me to this day. I got up when I heard the household really moving. Mrs Rea, always a charming hostess, suggested that I should have breakfast, but I said I would wait for Mr Rea. 'Oh! Carl won't be up yet'—and he wasn't, until about 11 o'clock, obviously with an early morning feeling. Boiled eggs were for breakfast and the eggs had on them cosies in the semblance of male fowl. The first thing Rea did was to take the one from his egg and exclaiming 'Damn silly things!' fling it across the room. He then savagely attacked the egg, which he expected to be hard-boiled, and spattered his tie and white waistcoat with yolk. He raged and stormed, his mildest remark being that the cook couldn't even boil an egg, then, catching my eye, did a grin, and entered into normal conversation, but with an occasional reference as to how eggs should be cooked.

The first morning at a Foray occasionally produced sparks if the waitress had not been previously coached properly by Mrs or Violet Rea. There was to be no porridge and tea was to be served at once with a jug of cold water. I've seen him terrify a girl out of her wits on the first day, but almost always nothing could be too good for him by the end of his stay. It was the same with the servants at home; they invariably adored him. Another

source of annoyance was butter. A 'sandwich list' always had 'Ham, no butter, no mustard' for him. If he got the wrong packet, or his instructions had not been carried out, away went the sandwich.

In the field he was the most patient leader of a Foray. Knowing with what little gladness he suffered fools, I was always struck with the manner in which he would name the same common species time after time throughout a long day. He was only irritable when someone queried a determination from lack of knowledge. As I always spent a good part of the time in his company, I saw many bubbles burst but never those blown by beginners.

One of the amusing things about the early Forays concerned tea. Rea never made any arrangements for tea, and Mrs Rea was always being appealed to for some modification of the programme so that this function could be achieved. Nothing in those days would persuade him to break off his collecting, let alone make a detour while tea was being organized. He called me a backslider and other things, but as years passed he became less spartan in this matter and in the end was as keen as anyone after a day in the woods.

To be with him in the haunts he had known from boyhood was always delightful. He would take one to the spot where some rarity grew, and he knew the plants, the birds, the insects; also the local traditions, the history, the archaeology. The district round Worcester was in his blood, and I got to know just how to draw him out on some subject when fungi were scarce, or we were on other matters bent.

He could be as stubborn as a mule and as cantankerous. When he got started off by something he resented, it was difficult to pull him up and he would sulk and fume in open meetings. Much of this was, I am sure, due to the fact that he had been spoiled in his younger days, for at bottom he was exceedingly kind and by nature courteous. I was often introduced by him to local farmers or anyone interested in natural history, and he would ask any youngster who showed signs of becoming a naturalist, to meet me at his home.

I cannot recollect ever sending him a specimen to name, though I sent him occasional special finds, but I made the most of every opportunity of collecting with him. He had an exceptional knowledge of fungi in the field, especially of Basidiomycetes, though he knew Discomycetes well and dabbled quite extensively in other groups. His writings, most of which appeared in our *Transactions*, are concerned mainly with the description of new or rare species but his knowledge of general mycology was considerable.

In his study he used an old-fashioned binocular Dollond microscope, which he kept under a glass shade. The assembling of the microscope and implements resembled some ritual, but time was eventually saved by the fact that his magnification was so arranged that one division in the scale of his eye-piece measured 1 μ.

When he undertook to write *British Basidiomycetae* we agreed to collaborate. He wrote the descriptions which I checked or emended from the Museum collection of original drawings and other sources. He pushed ahead at a great rate, partly because of temperament, partly because of

constant pressure by certain members. When I left for Macedonia I withdrew from collaboration, but helped considerably on my return. I should have preferred that, having the whole written up, there should have been a period of checking with specimens in the field and the insertion of original measurements where these were lacking. The descriptions were based on those of Fries and Quélet, whose works he always used, and collated with Mrs Rea's paintings. Consequently it has occasionally been questioned whether or not he really knew certain species; thus E. J. Gilbert doubts whether he had collected *Boletus sulphureus*, which happens to be one of the fungi I saw several times in his company, and which, incidentally, is far from rare.

Another point to remember is that if a varietal name had been given to a form he used it. 'It has a name' he would say, though he would admit that it was unworthy of denomination.

The book will remain a standard one. It has many imperfections, but these are far fewer than some would have us understand. In some ways it marks the end of a period, in others the beginning of another. It was not the best of which Rea was capable, but it is surprising that he accomplished it.

The older mycologists considered that he was too prone to follow the French school. It is more true to say that he regarded the work of Quélet, Boudier, and later, Réne Maire, as being more sound than that of most of the British mycologists of his period, and in this he was right. He was somewhat critical of the more modern French school, for he considered that it placed too much insistence on microscopic characters. His objection was probably mainly psychological. He knew his larger fungi as well as any man and he felt that the introduction of some of the new viewpoints based on indoor work, would lead to confusion, and ruin what he considered to be well established.

He collected in most parts of the British Isles but his visits abroad were few. He attended the meeting of the Société Mycologique de France in 1902 in company with C. B. Plowright. It was on this occasion that he was reasonably puzzled by the smell of *Phallus imperialis* being described, as he thought, as that of a church (odeur d'église) whereas it was of liquorice (réglisse): he attended a second meeting in 1930, and was elected an Honorary Member in 1934.

He was delighted to receive an invitation to attend the International Congress of Plant Sciences at Ithaca in 1926. He thoroughly enjoyed the trip across with about sixteen botanists, and was active in the ways available aboard ship. The Meetings and the trip to Yellowstone Park always stood out in his memory and he often referred to them. We took different journeys afterwards and gave our impressions of the American fungus flora in a short paper in the *Transactions* (XIV, 233–9). These did not refer to the fact that the first two American fungi I found were *Clavaria luteoalba* Rea and *Hygrophorus reai*! We were strolling through Enfield Glen with C. H. Kauffman. He and Rea were discussing some point and we stopped at a bend in the path for further directions. Almost at the spot where we were standing I picked up the two fungi and handed them to Rea. He didn't

move an eyelid, much to Kauffman's astonishment, when he learned what they were, but afterwards he laughingly asked me 'Did you see old Kauffman's face? He thought we'd brought the damned things with us.'

Right to the end he was his old self, active, alert and jovial. He had ceased playing tennis during the war but would push a mower over a large lawn on the hottest summer day. He walked with his usual tirelessness the last time I saw him.

He lost his first wife after a trying illness in 1927. His daughter Violet (Mrs Astley Cooper) gave up her art studies and returned home and carried on with the fungus drawings. When she married in 1929 he kept cheerfully on, but as his sole accomplishment in the cooking line had been one successful rice pudding at the beginning of Mrs Rea's serious illness, and his knowledge of the rest of housewifery was at about this level, he was completely in the hands of hirelings, who though doubtless well intentioned, could not supply the companionship and restraining influence he really needed. We were all happy therefore, when in 1931 he married Miss Susan Hubard and became his old self again, for she was fond of the open and keen to accompany him on that calendar of jaunts for Lodden lillies, for nuts, for fungi, for all nature's local specialities in their due season.

His activities for the cause of natural history, particularly that of Worcestershire, were unending. He acted as Chairman of the Museum and Library Committee of Worcester for fifty years.

Rea was fond of sport. He gained his college colours at rugger and retained an interest in the game. Cricket also interested him and a day in his company at the Worcester ground gave an insight to the size and assortment of his acquaintanceship. He was passionately fond of billiards and had a table in his study. He played tennis with characteristic vigour until well over seventy. He was a keen theatre-goer, enjoyed a Music Hall or a Revue, and appreciated operas. He loved gardens, and when he died in a chair in his study he had a button-hole of his favourite rose 'Dainty Bess', from a plant my wife had given him, for both for her and our daughter he had a warm affection.

LIST OF MEMBERS

Corrected to 30 June 1948

Honorary Members

Dodge, Dr B. O., New York Botanical Garden, Bronx Park, New York 58, N.Y., U.S.A. (1946.)

Falck, Professor R., Tiberius House, Goldzweig, Palestine. (1946.)

Gäumann, Professor E., Eidgenössischen Technischen Hochschule, Universitätstrasse, Zürich, Switzerland. (1946.)

Heim, Roger, Sous-Directeur au Muséum d'Histoire Naturelle, 11 Rue de Médicis, Paris (6e), France. (1930.) (1946.)

Lister, Miss Gulielma, F.L.S., 871 High Road, Leytonstone, London, E. 11. (1903.) (1924.)

Maire, René, D.Sc., F.M.L.S., Professeur à la Faculté des Sciences de l'Université, Algiers, Algeria, N. Africa. (1907.) (1939.)

Pearson, Arthur A., F.L.S., Nutcombe House, Hindhead Road, Hindhead, Surrey. (1911.) (1946.)

Petch, T., B.A., B.Sc., North Wootton, King's Lynn, Norfolk. (1911.) (1941.)

Ramsbottom, J., O.B.E., Dr.Sc., M.A., F.L.S., British Museum (Natural History), Cromwell Road, South Kensington, London, S.W. 7. (1910.) (1945.)

Swanton, E. W., O.B.E., A.L.S., Museum House, High Street, Haslemere, Surrey. (1899.) (1947.)

Wakefield, Miss E. M., M.A., F.L.S., The Herbarium, Royal Botanic Gardens, Kew, Surrey. (1911.) (1941.)

Members

Adams, Rev. J. H., Landulph Rectory, Hatt, Saltash, Cornwall. (1919.)

Ainsworth, G. C., B.Sc., Ph.D., F.L.S., London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C. 1. (1931.)

Alaily, Y. A. S. El, Société Anonyme de Wadi Kom-Ombo, P.O. Box 738, Cairo. (1935.)

Alcock, Mrs N. L., M.B.E., F.L.S., 61 Holywell, Oxford. (1919.)

Allchin, J. P., A.I.C., Ministry of Health, Regional Blood Transfusion Service, 17 Highfield Road, Birmingham 15. (1942).

Allen, C. Winckworth, 4 Leinster Square, Rathmines, Dublin, Eire. (1946.)

Anderson, Olof, The Botanical Museum, The University, Lund, Sweden. (1947.)

Appleby, Miss J. C., B.Sc., Ph.D., Boots Pure Drug Co. Ltd., Research Department, Bacteriological Division, Oakfields Road, West Bridgford, Nottingham. (1947.)

Armitage, F. D., A.R.P.S., F.R.M.S., Bargrove Lodge, Boxmoor, Herts. (1942.)

Ashby, S. F., B.Sc., c/o Barclays Bank, 13 Station Parade, Kew Gardens, Richmond, Surrey. (1926.)

Ashour, W. El. D., Department of Mycology and Plant Pathology, Imperial College of Science and Technology, South Kensington, London, S.W. 7. (1946.)

Atkins, F. C., Yaxley, Peterborough, Northants. (1945.)

Atkins, R. H., 2 Mersey View, Garston, Liverpool 19. (1943.)

Austwick, P. K. C., Mount MacGregor, 29 Surrenden Crescent, Brighton 6, Sussex. (1946.)

Bacon, Mrs Alice, B.Sc., F.L.S., Technical College, Brighton, Sussex. (1938.)

Badcock, E. C., Forest Products Research Laboratory, Princes Risborough, Bucks. (1944.)

Baker, Professor K. F., University of California, 405 Hilgard Avenue, Los Angeles 24, California, U.S.A. (1946.)

Baker, R. E. D., B.A., Imperial College of Tropical Agriculture, Trinidad. (1944.)
Balfour-Browne, Mrs F. L., M.Sc., Department of Botany, British Museum (Natural History), Cromwell Road, South Kensington, London, S.W. 7. (1930.)
Bannan, Miss L., B.Sc., A.R.C.S., 8 Belgrave Road, Chorlton cum Hardy, Manchester. (1945.)
Barnes, B., D.Sc., Ph.D., F.L.S., Chelsea Polytechnic, London, S.W. 3. (1922.)
Barnes, Miss E. M., B.Sc., Sir W. Dunn School of Pathology, University of Oxford. (1943.)
Barr, Rev. Robert, T.D., M.A., The Manse, Neilston, Renfrewshire. (1918.)
Barrington, Dr F. J. F., 52 Harley Street, London, W. 1. (1901.)
Baruah, Hitendra Kumar, M.Sc., 5/1 A Kayatala Road, Rashbihari Avenue, P.O., Calcutta 29, India. (1938.)
Bates, G. R., Ph.D., c/o Agricultural Dept., P.O. Box 387, Salisbury, Southern Rhodesia. (1930.)
Batko, Dr S., The Herbarium, Royal Botanic Gardens, Kew, Surrey. (1944.)
Batts, C. C. V., B.Sc., Edinburgh and East of Scotland College of Agriculture, 13 George Square, Edinburgh 8. (1946.)
Bawden, F. C., M.A., Rothamsted Experimental Station, Harpenden, Herts. (1941.)
Beaumont, Albert, M.A., N.A.A.S., Quarry Dene, Weetwood Lane, Leeds 6 (1924.)
Bennett, Miss M., B.Sc. (Hort.), East Malling Research Station, nr. Maidstone, Kent. (1945.)
Bewley, W. F., C.B.E., D.Sc., Experimental and Research Station, Cheshunt, Herts. (1922.)
Biffen, Professor Sir Rowland H., M.A., F.R.S., 136 Huntingdon Road, Cambridge. (1899.)
Biggs, Miss Rosemary, B.Sc., Ph.D., M.B., B.S., The Radcliffe Infirmary, Oxford. (1945.)
Birkinshaw, J. H., D.Sc., Firgrove, 4 Nower Hill, Pinner, Middlesex. (1946.)
Bisby, Guy R., Ph.D., Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey. (1921.)
Blackman, Professor V. H., M.A., Sc.D., F.R.S., F.L.S., 17 Berkeley Place, Wimbledon, London, S.W. 19. (1900.)
Blackwell, Miss Elizabeth Marianne, M.Sc., F.L.S., Botanical Department, Royal Holloway College, Englefield Green, Surrey. (1917.)
Blatchley, C. F. P., Ark Royal, St Helens Wood, St Leonards-on-Sea, Sussex. (1946.)
Blumer, Dr S., 9 Frohmatt Strasse, Waedenswil, Switzerland. (1936.)
Bollard, E. G., The Botany School, Cambridge. (1946.)
Bond, T. E. T., Ph.D., The University, Sheffield, Yorks. (1945.)
Boughey, A. S., B.Sc., Ph.D., Department of Botany, University College, Exeter. (1945.)
Bourgin, Dr Viennot, 8 Square Vauban, Viroflay, Seine-et-Oise, France. (1936.)
Boyd, A. E. W., B.Sc., Ph.D., Seed Testing, Plant Registration and Plant Pathology Station, Corstorphine, Edinburgh 12. (1947.)
Bradley Jones, J., N.A.A.S., Plant Pathology Department, University College, Cathays Park, Cardiff. (1948.)
Braid, Professor K. W., B.A., B.Sc., West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C. 2. (1922.)
Brenchley, G. H., M.A., N.A.A.S., Staplake Mount, Star Cross, Devon. (1925.)
Brett, Miss M., M.Sc., Ph.D., F.L.S., 9 Ox Lane, Harpenden, Herts. (1921.)
Brian, P. W., M.A., Ph.D., Imperial Chemical Industries Ltd., Butterwick Laboratories, The Frythe, Welwyn, Herts. (1944.)
Brierley, Professor W. B., D.Sc., F.R.A.I., F.L.S., Department of Agricultural Botany, The University, Reading. (1919.)

Broadfoot, Miss J., B.Sc., c/o Department of Agriculture and Lands, P.O. Box 387, Salisbury, Southern Rhodesia. (1945.)
Brooke, John, Westlea, Wormley, Herts. (1947.)
Brookfield, Miss E., 36A Fourth Avenue, Linden, Johannesburg, South Africa. (1946.)
Brooks, Professor F. T., C.B.E., M.A., F.R.S., F.L.S., The Botany School, Cambridge. (1907.)
Brown, Professor W., M.A., D.Sc., F.R.S., Imperial College of Science and Technology, South Kensington, London, S.W. 7. (1922.)
Buchwald, Professor N. F., Royal Agricultural and Veterinary College, Rulighedsvej 23, Copenhagen V, Denmark. (1946.)
Buckley, W. D., 'St Anthony', Leigh Park, Datchet, Bucks. (1916.)
Buddin, W., M.A., N.A.A.S., Chiltern Court, St Peter's Avenue, Caversham, Reading. (1921.)
Bunting, R. H., F.L.S., 3 Stanton Court, Weymouth. (1921.)
Burges, Professor N. A., The University, Sydney, N.S.W., Australia. (1935.)
Burman, A. D., 41 Trinley Road, Knightswood, Glasgow, W. 3. (1947.)
Burnett, J. H., M.A., Department of Botany, The University, Oxford. (1948.)
Burr, S., M.Sc., Department of Agriculture, The University, Leeds. (1924.)
Caldwell, Professor J., D.Sc., Ph.D., Department of Botany, University College, Exeter. (1932.)
Callen, E. O., B.Sc., Ph.D., F.L.S., Department of Plant Pathology, Macdonald College, McGill University, Quebec, Canada. (1941.)
Campbell, A. H., B.Sc., Ph.D., Glaxo Laboratories Ltd., Barnard Castle, Co. Durham. (1934.)
Canter, Mrs H., B.Sc., Freshwater Biological Association, Wray Castle, Ambleside, Westmorland. (1944.)
Carrothers, E. N., British Railways, York Road Station, Belfast. (1925.)
Carter, Mrs M., The Hydro, Goathland, Yorks. (1946.)
Cartwright, K. St G., M.A., F.L.S., The Old Vicarage, Towersey, Thame, Oxon. (1913.)
Catsimbas, C., B.Sc., Department of Botany and Plant Pathology, Imperial College of Science and Technology, South Kensington, London, S.W. 7. (1947.)
Cayley, Miss Dorothy M., Foxhall Cottage, Kelshall, nr. Royston, Herts. (1913.)
Charlton, Mrs K. M., Imperial Chemical Industries Ltd., Hawthorndale Laboratories, Jealott's Hill Research Station, Bracknell, Berks. (1945.)
Cheal, W. F., Botley Experimental Station, Botley, Hants. (1927.)
Chesters, Professor C. G. C., B.Sc., M.Sc., Ph.D., University College, University Park, Nottingham. (1930.)
Christensen, Dr Clyde M., University Farm, St Paul 8, Minn., U.S.A. (1945.)
Ciferri, Professor Dr R., Assistant Director, Laboratorio Crittogamico, Casella Postale 165, Pavia, Italy. (1926.)
Cleland, J. Burton, M.D., Professor of Pathology, University of Adelaide, South Australia. (1918.)
Clouston, D., M.A., D.Sc., North of Scotland Agricultural College, 41½ Union Street, Aberdeen. (1931.)
Colhoun, J., B.Sc., M.Agr., Ph.D., D.I.C., Plant Disease Division, Ministry of Agriculture, Queen's University, Belfast, Northern Ireland. (1943.)
Cook, W. R. I., B.Sc., Ph.D., Department of Botany, University College, Newport Road, Cardiff. (1924.)
Cooper, Mrs V. Astley, The Gables, Barham, Wakefield, Yorks. (1921.)
Corner, E. J. H., M.A., F.L.S., c/o Heffers, Cambridge. (1924.)
Cotton, Arthur D., O.B.E., V.M.H., F.L.S., Old Weavers, Pitchcoombe, nr. Stroud, Glos. (1902.)

Curtis, Miss Kathleen M., M.A., D.Sc., D.I.C., F.L.S., Mycologist, Biological Department, Cawthron Institute of Scientific Research, Nelson, New Zealand. (1917.)

Dade, H. A., A.R.C.S., Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey. (1927.)

Dale, W. T., B.Sc., Imperial College of Tropical Agriculture, Trinidad. (1944.)

Damiano, C., 81 Exeter House, Putney Heath, London, S.W. 15. (1946.)

Darimont, F., Assistant de botanique systematique à l'Université, Rue des Fontaines 92, Vottem (Liège), Belgium. (1947.)

Davies, D. L. G., Ph.D., N.A.A.S., Crosswood, Aberystwyth, Cards. (1938.)

Davies, D. W., B.Sc., N.A.A.S., Crosswood, Aberystwyth, Cards. (1923.)

Davies, H. M., B.Sc., Ph.D., 9 Queen's Road, Richmond, Surrey. (1944.)

Day, W. R., B.A., B.Sc., Imperial Forestry Institute, Oxford. (1928.)

Deacon, Dr G. E., Brundall, Norwich. (1933.)

Deighton, F. C., M.A., Mycologist, Department of Lands and Forests, Freetown, Sierra Leone, West Africa. (1925.)

Dennis, R. W. G., Ph.D., The Herbarium, Royal Botanic Gardens, Kew, Surrey. (1932.)

Dickinson, S., Ph.D., School of Agriculture, Cambridge. (1921.)

Dobbs, C. G., B.Sc., Ph.D., University College of North Wales, Department of Botany, Memorial Buildings, Bangor. (1933.)

Dodge, Dr Carroll W., Missouri Botanical Garden, 2315 Tower Grove Avenue, St Louis 10, Missouri, U.S.A. (1926.)

d'Oliveira, Professor B., Laboratorio de Patologia Vegetal, Instituto Superior de Agronomia, Tapada da Ajuda, Lisboa, Portugal. (1939.)

Dovaston, H. F., West of Scotland Agricultural College, Auchincruive, Ayr. (1946.)

Dowson, W. J., M.A., D.Sc., The Botany School, Cambridge. (1920.)

Duddington, C. L., M.A., F.L.S., Biology Department, The Polytechnic, 309 Regent Street, London, W. 1. (1946.)

Duncan, J. T., London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C. 1. (1930.)

Dunston, Capt. Ambrose E. A., Burltons, Donhead St Mary, nr. Shaftesbury, Dorset. (1937.)

Dunston, T. F. G. W., Burltons, Donhead St Mary, nr. Shaftesbury, Dorset. (1947.)

Elliot, Mrs J. S. Bayliss, D.Sc. (B'ham.), B.Sc. (London), Dulverton, Manor Way, Aldwick Bay, nr. Bognor Regis, Sussex. (1911.)

Ellis, E. A., F.L.S., Castle Museum, Norwich. (1937.)

Ellis, M. B., B.Sc., Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey. (1945.)

Emerson, R., Department of Botany, University of California, Berkeley, California, U.S.A. (1938.)

English, Miss M. P., M.Sc., Department of Botany, The University, Edgbaston, Birmingham 15. (1943.)

Eyre, Miss J. C., Wellbottom Lodge, Moulton, nr. Newmarket. (1915.)

Ferns, J. F., M.Sc., A.I.C., 16 Lawn Crescent, Kew, Surrey. (1942.)

Findlay, W. P. K., D.Sc., A.R.C.S., Forest Products Research Laboratory, Princes Risborough, Bucks. (1928.)

Fisher, S. D. P., Sackville Street, Leeds. (1930.)

Fitzpatrick, Professor H. M., Ph.D.; Plant Pathology Department, Cornell University, Ithaca, N.Y., U.S.A. (1935.)

Fletcher, Mrs P. T., B.A., 46 Neville's Court, Dollis Hill Lane, London, N.W. 2. (1935.)

Fletcher, W. W., B.Sc., Department of Botany, University of Glasgow. (1948.)

Foister, C. E., B.A., Ph.D., Plant Pathology Service, Seed Testing Station, East Craigs, Corstorphine, Edinburgh 12. (1940.)
Forsyth, Miss E. M. E., Biology Department, University College, Swansea, S. Wales. (1947.)
Fothergill, P. G., B.Sc., Ph.D., King's College, Newcastle-on-Tyne 2. (1946.)
Fraser, Miss Lilian R., D.Sc., Biological Branch, Dept. Agriculture, Box 36A, G.P.O., Sydney, Australia. (1938.)
Fries, Dr Nils, Institute of Physiological Botany, The University, Uppsala, Sweden. (1946.)
Gadd, C. H., D.Sc., Tea Research Institute, St Coombs, Talawakelle, Ceylon. (1921.)
Garrett, S. D., M.A., D.I.C., Department of Agriculture, Kingston, Jamaica, B.W. Indies. (1936.)
Gates, L. F., B.A., 115 Ingram Road, Thornton Heath, Surrey. (1947.)
Gemmell, A. R., B.Sc., Ph.D., M.S., Department of Botany, The University, Manchester 13. (1946.)
Gentles, J. C., B.Sc., Department of Botany, University of Glasgow. (1948.)
Gilbert, E. J., Docteur en Pharmacie, 4 Rue de Musset, Paris (16e), France. (1924.)
Gill, G. E., LL.B., Law Library, Four Courts, Dublin. (1937.)
Gillespie, J., B.Sc. (1938.)
Glynn, E. St Aubyn, 6A Lancaster Drive, London, N.W. 3. (1946.)
Glynne, Miss Mary D., D.Sc., Rothamsted Experimental Station, Harpenden, Herts. (1932.)
Goidanich, Dr Professor Gabriele, Stazione di Patologia Vegetale, Via Suzanna 13, Rome, Italy. (1947.)
Goodman, R. E. J., Norfolk Flax Establishment, Flitcham Abbey, Flitcham, King's Lynn, Norfolk. (1944.)
Graddon, W. D., B.Sc., A.M.I.Chem.E., The Brooms, Park Lane, Congleton, Cheshire. (1942.)
Grainger, Miss J., Wilshaw Road, Meltham, nr. Huddersfield. (1943.)
Grainger, J., B.Sc., Ph.D., Department of Plant Pathology, West of Scotland Agricultural College, Auchincruive, Ayr. (1944.)
Gray, Miss E. G., B.Sc., Ph.D., North of Scotland Agricultural College, 41½ Union Street, Aberdeen. (1942.)
Greenwood, E. L., Greenside, The Settlement, Ockbrook, Derby. (1944.)
Gregg, Miss Mary, B.Sc., Department of Botany, The University, Edgbaston, Birmingham 15. (1948.)
Gregory, P. H., Ph.D., Rothamsted Experimental Station, Harpenden, Herts. (1930.)
Grenfell, Miss E., B.Sc., 23 Ashley Road, Epsom. (1947.)
Griffiths, Ellis G., Agricultural Buildings, Alexandra Road, Aberystwyth. (1948.)
Groves, Dr J. Walton, Central Experiment Farm, Ottawa, Canada. (1942.)
Gwynne, D. C., N.A.A.S., Plant Pathology Department, Elswick Hall, Elswick Park, Newcastle-on-Tyne 4. (1946.)
Gwynne-Vaughan, Professor Dame Helen, G.B.E., D.Sc., LL.D., F.L.S., 93 Bedford Court Mansions, London, W.C. 1. (1906.)
Hainsworth, E., M.A., Tocklai Experimental Station, Cinnamara, P.O. Assam, India. (1946.)
Hall, Rev. Charles A., F.R.M.S., 'Clynder', 71 Landsdowne Road, Worthing. (1948.)
Hanna, W. F., M.Sc., Ph.D., c/o University of Manitoba, Fort Garry, Manitoba, Canada. (1925.)
Hansford, C. G., M.A., F.L.S., Dartonfield, P.O. Agalawatta, Ceylon. (1921.)
Harley, J. L., M.A., D.Phil., Department of Botany, The University, Oxford. (1932.)
Harris, G. C. M., M.A., D.Phil., Imperial Chemical Industries Ltd., Dyestuff Division, Blackly, Manchester. (1946.)

Harris, R. V., D.Sc., A.R.C.S., East Malling Research Station, nr. Maidstone, Kent. (1924.)
Harrison, T. H., D.Sc., 24 Ray Road, Epping, N.S.W., Australia. (1931.)
Hart, H. G., Royal Societies Club, 35 Dover Street, London, W. 1. (1946.)
Haskins, Major R. H., The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge 38, Mass., U.S.A. (1946.)
Hassell, F. C., 8 Sunbury Gardens, Dartry Road, Dublin, S. 2, Eire. (1946.)
Hastings, Somerville, M.S., F.R.C.S., 12 Westminster Palace Gardens, Victoria Street, London, S.W. 1. (1913.)
Hawker, Miss L. E., Ph.D., D.Sc., Botanical Department, The University, Bristol. (1934.)
Hawkey, Miss Betty, 335 West Wycombe Road, High Wycombe, Bucks. (1948.)
Hay, L. A., 76 Hopper Street, Wellington, New Zealand. (1946.)
Heimbeck, Mrs Louise, Brosoe, Levanger, Norway. (1923.)
Hemming, H. G., Imperial Chemical Industries Ltd., Butterwick Laboratories, The Frythe, Welwyn, Herts. (1946.)
Hendrickx, F. L., Mulungu Experiment Station, I.N.E.A.C., Mulungu, Belgian Congo. (1946.)
Herd, Miss S. J., B.Sc., Chalk Lane Hotel, 1 Woodcote End, Epsom, Surrey. (1947.)
Hewett, P. D., 61 Chester Drive, North Harrow, Middlesex. (1947.)
Hickman, C. J., B.Sc., M.Sc., Ph.D., Botanical Department, The University, Edgbaston, Birmingham 15. (1935.)
Hilton, R. N., Clare College, Cambridge. (1948.)
Hiscott, G. S., Furzy Grove, Kempshott Lane, Basingstoke, Hants. (1944.)
Hitchcock, Miss M. M., East Malling Research Station, nr. Maidstone, Kent. (1945.)
Hof, Dr T., Centraal Instituut Voor Materiaalonderzoek, Rijswijk (Bij Delft), Lange Kleiweg 22, Holland. (1947.)
Holden, H. S., D.Sc., F.R.S.E., F.L.S., Metropolitan Police Laboratory, New Scotland Yard, London, S.W. 1. (1923.)
Holmes, G. D., University College of North Wales, Bangor, Caern. (1947.)
Hora, Dr F. B., The University, Reading, Berks. (1943.)
Howard, H. J., F.R.M.S., F.L.S., Lingfield, 6 College Road, Norwich. (1918.)
Howe, F. J., F.R.M.S., Pathological Department, St Cross Hospital, Rugby, Warwickshire. (1943.)
Hughes, G. C., 20 Priory Road, Bicester, Oxon. (1898.)
Hughes, J. S., M.A., 65 George Street, Summertown, Oxford. (1927.)
Hughes, S. J., M.Sc., Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey. (1941.)
Huijsmann, Dr H. S. C., Oogarts, Doetinchen, Holland. (1947.)
Hull, R., B.Sc., Ph.D., A.R.C.S., c/o Rothamsted Experimental Station, Field Research Station, Hackthorn, Lincoln. (1942.)
Hurst, C. P., F.L.S., Landulph Rectory, Saltash, Cornwall. (1928.)
Hutchinson, Stephen A., Ph.D., Department of Botany, The University, Glasgow, (1948.)
Ingold, Professor C. T., D.Sc., Ph.D., F.L.S., Department of Botany, Birkbeck College, Chancery Lane, London, E.C. 4. (1935.)
Isaac, I., B.Sc., Ph.D., The Grammar School, Cheltenham, Glos. (1943.)
Jacks, H., Botany Department, Imperial College of Science and Technology, South Kensington, London, S.W. 7. (1947.)
Jackson, R. M., North of Scotland Agricultural College, 41½ Union Street, Aberdeen. (1946.)
Jamieson, Miss M. M., Imperial Chemical Industries Ltd., Butterwick Laboratories, The Frythe, Welwyn, Herts. (1947.)
Jay, B. A., M.A., F.L.S., The Quinta, 19 Elm Close, Amersham, Bucks. (1946.)

Jeffreys, E. G., Imperial Chemical Industries Ltd., Butterwick Laboratories, The Frythe, Welwyn, Herts. (1946.)
Jenkins, Miss Anna E., Bureau of Plant Industry, Department of Agriculture, Washington, D.C., U.S.A. (1942.)
Johnstone, R. H., 726 Anniesland Road, Glasgow, W. 4. (1938.)
Jones, D. K., M.Sc., 39 Queens Road, Aberystwyth, Cards. (1947.)
Jørstad, Ivar, Statsmykolog, Botanisk Museum, Oslo, Norway. (1923.)
Justham, Mrs M. C. D., Ph.D., N.A.A.S., Bracken Hill, Leigh Woods, Bristol 8. (1945.)
Keay, Miss M. A., M.A., Ph.D., Imperial Bureau of Plant Breeding and Genetics, School of Agriculture, Cambridge. (1935.)
Kelsall, Mrs H. M., 68 Harborne Road, Birmingham 15. (1947.)
Kent, W. W., Red House, Rodbourne, Swindon, Wilts. (1948.)
Keyworth, W. G., Ph.D., D.I.C., East Malling Research Station, nr. Maidstone, Kent. (1941.)
King, J. M. B., 23 Lyncroft Gardens, Ealing, London, W. 13. (1945.)
Klein, Richard, Ph.D., F.L.S., 3 Sussex Place, London, W. 2. (1943.)
Knight, Major Maxwell, O.B.E., The Homestead, Park Road, Camberley, Surrey. (1945.)
Knight, Mrs S., The Homestead, Park Road, Camberley, Surrey. (1945.)
Korf, R. P., Department of Plant Pathology, Cornell University, Itchaca, N.Y., U.S.A. (1947.)
Kramer, L. M. J., M.A., Ph.D., 41 Marlborough Mansions, Cannon Hill, London, N.W. 6. (1946.)
Krebs, Mrs Lottie, 42 Selcroft Road, Purley, Surrey. (1948.)
Lane, C. J., 34 Lavender Gardens, London, S.W. 11. (1947.)
Large, E. C., 56 Church Lane, Girton, Cambs. (1948)
La Touche, C. J., M.Sc., L.A.H., Mushroom Research Association, Yaxley, nr. Peterborough, Northants. (1946.)
Leach, R., B.A., Byrns Lodge, Grantchester, Cambridge. (1929.)
Lee, O. M., School of Agriculture, Serdang, Selangor, Malaya. (1946.)
Legge, Miss B. J., B.Sc., Ph.D., Department of Biology, University College, Singleton Park, Swansea. (1943.)
Levisohn, Miss I., D.Phil., Forestry School, 6 Keble Road, Oxford. (1943.)
Line, James, M.A., School of Agriculture, Cambridge. (1921.)
Lloyd, Dr Blodwen, Royal Technical College, Glasgow, C. 1. (1946.)
Loader, Miss F. M., B.Sc., Botanical Department, University College, Southampton. (1927.)
Longley, B., Slape Mills, Netherbury, nr. Beaminster, Dorset. (1946.)
Lumb, M., 18 Chaworth Road, West Bridgford, Nottingham. (1945.)
Lütjeharms, Professor W. J., Phil.Nat.D., Department of Botany, University College, Bloemfontein, Orange Free State, S. Africa. (1930.)
Macdonald, James A., Botany Department, The University, St Andrews. (1938.)
McKay, Robert, B.Sc., A.R.C.S.I., Department of Plant Pathology, Albert Agricultural College, Glasnevin, Dublin, N.W. 3. (1939.)
McKee, R. K., Midland Agricultural College, Sutton Bonington, Loughborough, Leics. (1947.)
Mackinnon, Juan E., M.D., Instituto de Higiene, Av. Ramón Anador, 3051 Parque J. Batlle y Ordonez, Montevideo, Uruguay. (1947.)
Madge, Margaret A. P., Ph.D., Royal Holloway College, Englefield Green, Surrey. (1948.)
Majolier, Mrs N., 288 King's Road, Chelsea, London, S.W. 3. (1946.)
Malençon, G., Inspecteur Principal, Bureau de la Dèfense Végétaux, 65 bis Avenue de Témara, Rabat, Morocco. (1946.)

Marsh, R. W., M.A., Research Station, Long Ashton, Bristol. (1923.)
Martens, Professor P., 23 Rue Marie-Thérèse, Louvain, Belgium. (1947.)
Martin-Scott, I., M.D., 16 Buckingham House, Courtlands, Richmond, Surrey. (1948).
Masefield, G. B., c/o Department of Agriculture, Entebbe, Uganda. (1932.)
Mason, E. W., M.A., M.Sc., F.L.S., Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey. (1921.)
Mason, Mrs E. W., M.Sc., Inglenook, 63 King's Road, Richmond, Surrey. (1922.)
Mathias, W. T., B.Sc., The University, Liverpool. (1938.)
Mathieson, Miss M. J., M.Sc., Botany School, Cambridge. (1947.)
Matthews, Professor J. R., M.A., F.L.S., Department of Botany, The University, Old Aberdeen. (1921.)
Maxted, Mrs B., B.Sc., Ph.D., Department of Botany, The University, Reading. (1934.)
Mehta, Professor K. C., Ph.D., Department of Biology, Agra College, Agra, U.P., India. (1921.)
Melin, Professor J. B. E., Institute of Physiological Botany, Uppsala, Sweden. (1946.)
Miller, Professor J. H., B.S., M.S., Ph.D., University of Georgia, Athens, Ga., U.S.A. (1930.)
Molina-Liarden, M., Plant Pathology Department, Cornell University, Ithaca, N.Y., U.S.A. (1947.)
Montague, Mrs A., Penton, Crediton, N. Devon. (1898.)
Montgomery, H. B. S., B.A., Ph.D., D.I.C., East Malling Research Station, nr. Maidstone, Kent. (1940.)
Moore, Miss E. M., Department of Plant Pathology, School of Agriculture, University of Wisconsin, Madison 6, Wis., U.S.A. (1943.)
Moore, Miss F. J., Ph.D., Ministry of Agriculture, Plant Pathology Laboratory, Milton Road, Harpenden, Herts. (1945.)
Moore, M. H., M.Sc., East Malling Research Station, nr. Maidstone, Kent. (1940.)
Moore, W. C., M.A., Ministry of Agriculture, Plant Pathology Laboratory, Milton Road, Harpenden, Herts. (1922.)
Morgan, Miss J. P., University College, Nottingham. (1947.)
Morris, L. E., c/o Eton College, Windsor, Berks. (1924.)
Moss, P. S., Box 3, P.O., Townsville, Queensland, Australia. (1947.)
Moureau, Dr J., Laboratoire de Phytopathologie, I.N.E.A.C., Yangambi, Belgian Congo. (1944.)
Munson, R. G., 15 Munro Gardens, Harrow Weald, Middlesex. (1943.)
Muskett, Professor A. E., D.Sc., A.R.C.S., M.R.I.A., Queen's University, Belfast, Northern Ireland. (1923.)
Nannfeldt, Professor J. A., Sturegatan 11, Uppsala, Sweden. (1932.)
Nash-Wortham, J. R. H., 19 Grand Parade, Brighton. (1937.)
Nattrass, R. M., B.Sc. (Agric.), Ph.D., Department of Agriculture, P.O.B. 338, Nairobi, Kenya. (1925.)
Newby, H. V., Department of Botany, Queen Mary College, London, E. 1. (1946.)
Newhook, F. J., M.Sc., Department of Mycology and Plant Pathology, Imperial College of Science and Technology, London, S.W. 7. (1946.)
Nobbs, Miss G. E. D., 4 Clifton Estate, Plympton, S. Devon. (1943.)
Noble, Miss Mary, B.Sc., Ph.D., 19A Willowbrae Avenue, Edinburgh. (1940.)
Noel, Miss E. F., F.L.S., 37 Burnham Court, Queen's Road, London, W. 2. (1913.)
O'Connor, P., Ph.D., B.Sc., A.R.C.Sc.I., National Museum, Dublin. (1925.)
Ogilvie, L., M.A., M.Sc., N.A.A.S., Bracken Hill, Leigh Woods, Bristol 8. (1922.)
Olliver, C. W., c/o Lloyds Bank Ltd., 50 Notting Hill Gate, London, W. 11. (1942.)
Orton, P. D., M.A., A.R.C.M., Epsom College, Epsom, Surrey. (1947.)
Osborne, J. F., 21 Grimsdyke Road, Hatch End, Middlesex. (1944.)

Padwick, Dr G. Watts, Imperial Chemical Industries Ltd., Nobel House, 2 Buckingham Gate, London, S.W. 1. (1936.)
Page, Miss W. M., M.Sc., Ph.D., 5 Dartmouth Chambers, Theobald's Road, London, W.C. 1. (1921.)
Parker, Professor C. S., Department of Botany, Howard University, Washington, D.C., U.S.A. (1932.)
Paton, Miss M. R., Hareshawmuir by Kilmarnock, Ayrshire. (1945.)
Peach, Mrs Mary, 5 Tybenham Road, Merton Park, London, S.W. 19. (1947.)
Peklo, Professor Dr Jaroslav, Institute of Phytopathology, College of Agriculture and Forestry, Prague XIX, Dejvice, Czechoslovakia. (1924.)
Pettifer, A. J., 36 Cheviot Gardens, Hendon Way, London, N.W. 2. (1944.)
Peyronel, Professor B., Directeur, Istituto di Patologia Vegetale, Via Pietro Giuria 15, Torino, Italy. (1932.)
Phillips, Dr H. H., 11 Kings End, Bicester, Oxon. (1923.)
Pilat, Dr A., Department of Botany, National Museum, Zahradní 44, Prague XIX, Czechoslovakia. (1946.)
Ping, A. Wentworth, M.A., 17 Grosvenor Terrace, Bootham, York. (1926.)
Plack, R. L., 44 Regent Road, Leicester. (1947.)
Platt, J., B.Sc., 54 Eldon Road, Cheltenham, Glos. (1947.)
Plunkett, B. E., B.Sc., Botany Department, Birkbeck College, London, E.C. 4. (1945.)
Porter, S. C., A.R.I.C., A.M.I.Chem.E., 42 Crofton Road, Ipswich, Suffolk. (1946.)
Prentice, I. W., B.Sc., Ph.D., East Malling Research Station, nr. Maidstone, Kent. (1943.)
Preston, N. C., B.Sc., N.A.A.S., Woodthorne, Wergs Road, Tettenhall, nr. Wolverhampton. (1920.)
Ramsbottom, Miss Mary, 34 Ennerdale Road, Richmond, Surrey. (1946.)
Rauter, Dr F., 33 Holmfield Avenue, London, N.W. 4. (1942.)
Ray, Miss Anne, Penarwyn, Gorran Haven, Gorran, Cornwall. (1929.)
Rayner, Dr M. Cheveley (Mrs Neilson Jones), Bedford College for Women, Regent's Park, London, N.W. 1. (1921.)
Reed, J., 41 Freegrove Road, London, N. 7. (1946.)
Rees, John, M.Sc., Advisory and Research Department in Agriculture, University College, Cardiff. (1929.)
Reichert, Dr Israel, Jewish Agency for Palestine, Agricultural Research Station, Rehovot, Palestine. (1924.)
Rhodes, Miss Mabel, Lister Institute, Chelsea Gardens, London, S.W. 1. (1921.)
Ritson, W., 12 West Street, Winwick Road, Warrington, Lancs. (1946.)
Roberts, Emyr T., B.Sc., A.R.C.S., N.A.A.S., Chiltern Court, St Peter's Avenue, Caversham, Reading, Berks. (1947.)
Robertson, N. F., West African Cacao Research Institute, Tafo, Gold Coast. (1945.)
Robinson, E., 26 Burwood Avenue, Eastcote, Pinner, Middlesex. (1938.)
Rogers, Dr D. P., New York Botanical Garden, Bronx Park, New York 58, N.Y., U.S.A. (1943.)
Rogerson, Clark T., Department of Plant Pathology, Cornell University, Ithaca, N.Y., U.S.A. (1947.)
Roscoe-Abbott, Capt. J., B.Sc., The Rest, Holme-next-Sea, King's Lynn, Norfolk. (1946.)
Rose, H. V., Morden College, Blackheath, London, S.E. 3. (1944.)
Roumain, P., Département de l'Agriculture, Damien, Port-au-Prince, Haiti. (1946.)
de Rousset-Hall, O., M.A., 44 Great Bowden Road, Market Harborough, Leics. (1942.)
Roy, R. Y., Benares, Hindu University, India. (1947.)

Sampson, Miss K., M.Sc., Malmsmead, Lacey Green, nr. Aylesbury, Bucks. (1920.)
Samuel, G., M.Sc., Rothamsted Experimental Station, Harpenden, Herts. (1923.)
Sansome, Mrs E. R., M.Sc., Botanical Department, The University, Manchester 13. (1945.)
Savory, J. G., B.Sc., Forest Products Research Laboratories, Princes Risborough, nr. Aylesbury, Bucks. (1946.)
Schärer-Bider, W., Peter Rot-Str. 58, Bâle, Switzerland. (1947.)
Schimmer, Miss F. C., M.Sc., Wye College, Ashford, Kent. (1943.)
Searle, G. Odell, B.Sc. (Agric.), Flax Research Institute, Flitcham Abbey, Flitcham, King's Lynn, Norfolk. (1920.)
Shapiro, A., Middlesex Colony, Harper Lane, Shenley, Herts. (1946.)
Shear, Dr C. L., U.S. Department of Agriculture, Bureau of Plant Industry, Washington, D.C., U.S.A. (1930.)
Sheard, Mrs E., Experimental and Research Station, Cheshunt, Herts. (1937.)
Sidky, S. T., D.I.C., Ph.D., Ministry of Agriculture, Mycological Department, Cairo, Egypt. (1947.)
Sisley, C., Cuprinol Department, Robbialac Paints, 36 St James's Street, London, S.W. 1. (1947.)
Skene, Professor Macgregor, Department of Botany, The University, Bristol. (1936.)
Smith, Alexander, M.A., Ph.D., Ministry of Agriculture, Plant Pathology Laboratory, Milton Road, Harpenden, Herts. (1924.)
Smith, Miss E. K. Goldie, Royal Holloway College, Englefield Green, Surrey. (1944.)
Smith, G., M.Sc., F.R.I.C., London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C. 1. (1936.)
Smith, Miss J. L., B.Sc., East Malling Research Station, nr. Maidstone, Kent. (1946.)
Smith, Professor Noel J. G., Ph.D., B.Sc., Botany Department, Rhodes University College, Grahamstown, S. Africa. (1924.)
Snow, Douglas, Ph.D., Department of Agricultural Botany, The University, Reading, Berks. (1947.)
Sowter, F. A., F.L.S., 9 North Avenue, Leicester. (1942.)
Sparrow, Dr F. K., Department of Botany, University of Michigan, Ann Arbor, Mich., U.S.A. (1946.)
Spilsbury, J. F., B.Sc., W.P.R.L., Langley Court, Beckenham, Kent. (1946.)
Stakman, Professor E. C., University of Minnesota, Department of Agriculture, University Farm, St Paul 8, Minn., U.S.A. (1922.)
Stephens, Miss E. L., B.A., Department of Botany, University of Cape Town, South Africa. (1928.)
Sterry, Miss M., B.Sc., Manor Cottage, Longhope, Glos. (1942.)
Stevenson, Dr J. A., U.S. Department of Agriculture, Bureau of Plant Industry, Plant Industry Station, Beltsville, U.S.A. (1927.)
Stewart, Miss D. D., British Drug Houses Ltd., City Road, London, N. 1. (1946.)
Steyaert, R. L., Ing.A.I.Gx., 159 Rue des Atrebates, Etterbeck, Brussels iv, Belgium. (1931.)
Stiles, Professor W., Sc.D., F.R.S., Botanical Department, The University, Edgbaston, Birmingham 15. (1936.)
Stirrup, H. H., M.Sc., Nottingham University College School of Agriculture, Sutton Bonington, nr. Loughborough, Leics. (1922.)
Storey, H. H., M.A., Ph.D., Colonial Office, Downing Street, London, S.W. 1. (1922.)
Storey, I. F., Ph.D., N.A.A.S., Quarry Dene, Weetwood Lane, Leeds 6. (1941.)
Stubbs, J., B.Sc., Ph.D., Imperial Chemical Industries Ltd., Hawthorndale Laboratories, Jealott's Hill Research Station, Bracknell, Berks. (1947.)
Talbot, P. B. H., Ph.D., P.O. Box 994, Pretoria, South Africa. (1945.)
Tervet, I. W., B.Sc., Agricultural Experiment Station, Lincoln, Nebraska, U.S.A. (1933.)

Thirumalachar, M. J., M.Sc., D.Sc., 20th V Main Road, Malleswaram, Bangalore, India. (1945.)
Thomas, D. G., Heathfield, Upper Tumble, Llanelly, Carmarthen. (1946.)
Thomas-Perrott, Mrs P. E., The Training College, Dudley, Worcs. (1945.)
Thompson, Miss E. C., East Malling Research Station, nr. Maidstone, Kent. (1942.)
Thompson, Miss Jeanne, B.Sc., Botanical Department, The University, Edgbaston, Birmingham 15. (1947.)
Thompson, Miss M. V., Pathological Research Laboratory, Christie Hospital and Holt Radium Institute, Manchester 20. (1942.)
Thurston, Miss J. M., B.Sc., Rothamsted Experimental Station, Harpenden, Herts. (1942.)
Tipler, R. V., B.Sc., A.R.C.S., Imperial Chemical Industries Ltd., Hawthorndale Laboratories, Jealott's Hill Research Station, Bracknell, Berks. (1945.)
Tolba, M. K., B.Sc., M.Sc. (Cairo), Imperial College of Science and Technology, South Kensington, London, S.W. 7. (1947.)
Tomkins, R. G., M.A., Ph.D., Trinity College, Cambridge. (1925.)
Topping, Mrs M. D., B.Sc., 32 Eastwood Drive, Littleover, Derby. (1933.)
Topping, Mrs M. P., Angroban, Fir Tree Road, Leatherhead, Surrey. (1947.)
Traaen, Professor A. E., Agricultural College, Vollebekk, Norway. (1946.)
Trouern-Trend, K., Standlynch Farmhouse, Trafalgar, Drinton, nr. Salisbury, Wilts. (1946.)
Tunstall, A. C., Tocklai Experimental Station, Cinnamara P.O., Assam, India. (1933.)
Turner, Miss E. M., B.A., Ph.D., Old School House, Great Haseley, Oxon. (1940.)
Turner, Mrs F. M., Rosecot, 26 Halls Road, Tilehurst, Reading, Berks. (1943.)
Turner, Mrs M., M.Sc., University College, University Park, Nottingham. (1945).
Twyman, E. S., B.Sc., M.Sc., Ph.D., Botanical Department, The University, Edgbaston, Birmingham 15. (1942.)
van-Beverwijk, Miss A. L., Eemnesserweg 42, Baarn, Holland. (1947.)
van Eyndhoven, G. L., Eindenhoutstraat 36, Haarlem, Holland. (1947.)
Vanterpool, T. C., M.Sc., Botanical Department, University of Saskatchewan, Saskatoon, Canada. (1929.)
Venkatarayan, S. V., Mycologist, Agricultural Department, Bangalore, S. India. (1935.)
Vines, Professor W. S., University College, Hull, E. Yorks. (1947.)
Wadham, Professor S. M., M.A., Department of Agriculture, The University, Melbourne, Victoria, Australia. (1922.)
Waldie, J. S. L., B.Sc., C.D.A., Department of Agricultural Botany, The University, Reading. (1925.)
Walker, A. G., Ph.D., N.A.A.S., Wye, nr. Ashford, Kent. (1946.)
Walker, Mrs J., London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C. 1. (1947.)
Walker, Mrs K. M., Strathmore Private Hotel, 25 Petersham Road, Richmond, Surrey. (1945.)
Wallace, E. R., M.A., 168 West Parade, Lincoln. (1934.)
Wallace, G. B., B.Sc. (Agric.), Ph.D., Lyamungu, Moshi, Tanganyika Territory, E. Africa. (1928.)
Warcup, J. H., M.Sc., The Botany School, Cambridge. (1947.)
Wardlaw, Professor C. W., Department of Cryptogamic Botany, The University, Manchester 13. (1943.)
Warne, Mrs M. M., B.A., 25 Amherst Road, Fallowfield, Manchester 14. (1937.)
Warren, Dr Clara M., M.R.C.S., L.R.C.P., Fiveways, Leyborne Park, Kew, Richmond, Surrey. (1946.)
Waterhouse, Miss G. M., M.Sc., 95 Knightwood Crescent, New Malden, Surrey. (1927.)
Waterston, J. M., B.Sc., Department of Agriculture, Moor Plantation, Ibadon, Nigeria, British West Africa. (1934.)

Watson, W., D.Sc., A.L.S., Cedene, Cheddon Road, Taunton, Som. (1923.)
Webb, Dr Phillip, The Yews, Chadlington, Oxford. (1938.)
Webb, R. A., B.A., D.Phil., National Hospital, Queen's Square, London, W.C. 1. (1936.)
Webster, J., B.Sc., Botany Department, University College, Hull. (1945.)
Westerdijk, Professor Johanna, Javalaan 4, Baarn, Holland. (1923.)
Western, J. H., B.Sc., Ph.D., N.A.A.S., Elswick Hall, Elswick Park, Newcastle-on-Tyne. (1934.)
Weston, W. A. R. Dillon, M.A., Ph.D., N.A.A.S., Anstey Hall, Trumpington, Cambridge. (1923.)
Whitaker, F. Owen, 51 Grosvenor Avenue, Carshalton, Surrey. (1921.)
White, Neville H., D.Sc., Faculty of Agriculture, The University, Sydney, New South Wales, Australia. (1944.)
White, Dr W. Lawrence, Biological Laboratories, Philadelphia Quatermaster Depot, 2800 South 20th Street, Philadelphia 45, Pa., U.S.A. (1947.)
Whitehead, T., D.Sc., A.R.C.S., School of Agriculture, University College of North Wales, Bangor, Caern. (1920.)
Wilkins, W. C., University College of North Wales, Bangor, Caern. (1947.)
Wilkins, W. H., M.A., D.Phil., Department of Botany, The University, Oxford. (1928.)
Wilkinson, E. H., B.Sc., Ph.D., Newnham Farms, Newnham Bridge, nr. Tenbury Wells, Worcs. (1938.)
Williams, P. H., B.Sc., Experimental and Research Station, Cheshunt, Herts. (1930.)
Wilson, Miss A. P., M.B.E., A.R.C.S., 7A Arlington Drive, Nottingham. (1929.)
Wilson, Alastair R., Ph.D., Nottingham University College, School of Agriculture, Sutton Bonington, Loughborough. (1933.)
Wilson, Miss Irene M., B.Sc., Ph.D., Botany Department, University College of Wales, Aberystwyth. (1938.)
Wilson, Malcolm, D.Sc., A.R.C.S., F.L.S., Royal Botanic Garden, Edinburgh. (1912.)
Wiltshire, S. P., D.Sc., Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey. (1920.)
Wolf, Dr F. A., Department of Botany, Duke University, Durham, North Carolina, U.S.A. (1940.)
Wolf, Dr F. T., Vanderbilt University, Nashville, Tennessee, U.S.A. (1940.)
Wood, F. C., The Rest, Franklin Road, Durrington, Worthing. (1935.)
Wood, R. K. S., Botany Department, Imperial College of Science and Technology, South Kensington, London, S.W. 7. (1946.)
Woodcock, A. J. A., M.Sc., F.E.S., Rhianva, 65 Rock Avenue, Gillingham, Kent. (1926.)
Woodward, R. C., Ph.D., Export Department, Plant Protection Ltd., Nobel House, Buckingham Gate, London, S.W. 1. (1924).
Wormald, H., D.Sc., A.R.S.C., East Malling Research Station, nr. Maidstone, Kent. (1921.)
Wright, F. R. Elliston, Braunton, N. Devon. (1946.)
Yuill, E., 31 Askham Lane, Ascomb, York. (1937.)

Associates

Beaumont, Miss B. S., 40 Lidgit Street, Lindley, Huddersfield, Yorks. (1947.)
Best, Miss E. J., Rathmines, 23 North Park Drive, Blackpool, Lancs. (1947.)
Bolton, Miss Margaret, Oakhurst, 3 Cecil Road, Weston-super-Mare, Somerset. (1947.)
Brokenshire, F. A., 2 Rock Avenue, Barnstaple, N. Devon. (1946.)
Brown, Miss Evelyn A., 8 Twyford Crescent, Acton, London, W. 3. (1947.)
Charlton, J. L., B.Sc., Dip. Bact., Imperial Chemical Industries Ltd., Hawthorndale Laboratories, Jealott's Hill Research Station, Bracknell, Berks. (1947.)

Chesters, Miss K. I. M., 101 Harlaxton Drive, Lenton Sands, Nottingham. (1947.)
Cook, Miss Jean M., 61 Frederick Road, Wylde Green, Sutton Coldfield, nr. Birmingham. (1947.)
Damiano, Mrs A. M., 81 Exeter House, Putney Heath, London, S.W. 15. (1946.)
Dobbs, Mrs C. G., 101 Gladstone Terrace, Bangor, Caern. (1947.)
Gover, Miss D. J., B.Sc., 49 Cotswold Road, Sheffield 6. (1945.)
Gray, Miss M. R., Royal Holloway College, Englefield Green, Surrey. (1947.)
Gregory, Mrs M. F., 39 Ox Lane, Harpenden, Herts. (1945.)
Harvey, Miss E. M., 12 King George Street, Greenwich, London, S.E. 10. (1946.)
Hunt, Professor H. J., Royal Holloway College, Englefield Green, Surrey. (1945.)
Jacobs, J. A., M.A., A.F.R.Ae.S., 10 Woodland Avenue, Windsor, Berks. (1946.)
James, L., 19 Bushey Road, Ickenham, Uxbridge, Middlesex. (1947.)
Kelsall, R. K., 68 Harborne Road, Birmingham 15. (1947.)
Lickfold, Miss K. E., 83 Largewood Avenue, Tolworth, Surbiton, Surrey. (1947.)
Lock, Miss H. M., 99 Thames Drive, Leigh-on-Sea, Essex. (1947.)
Lofts, Miss M., 14 Broomboro, Totnes, Devon. (1947.)
Majolier, C. M. P., 288 King's Road, Chelsea, London, S.W. 3. (1946.)
Martin, Miss J., B.Sc., 1 Gordon Road, Oundle, Peterborough, Northants. (1947.)
May, Miss K., Royal Holloway College, Englefield Green, Surrey. (1947.)
Mayer, Miss B. M., 171 Avery Hill Road, New Eltham, London, S.E. 9. (1946.)
Metcalfe, C. R., B.A., Ph.D., Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey. (1947.)
Molesworth, Miss B., Royal Holloway College, Englefield Green, Surrey. (1945.)
Moore, Mrs B., Vallis, Amenbury Lane, Harpenden, Herts. (1945.)
Moore, Mrs J. B., B.Sc., Ph.D., Juglans, 17 Conway Road, Maidstone, Kent. (1945.)
Noaks, Miss B. A., 25 Waldegrave Gardens, Twickenham, Middlesex. (1947.)
Osterburg, Miss V. M., 'Orlingbury', Lawn Avenue, West Drayton, Middlesex. (1946.)
Partridge, Miss B. M., Mycology Laboratory, Department of Botany, The University, Oxford. (1946.)
Read, Miss L. M., East Malling Research Station, nr. Maidstone, Kent. (1946.)
Rolinson, G. N., 7 Corby Road, Mapperley, Nottingham. (1948).
Salt, G. A., Department of Botany, The University, Oxford. (1946.)
Stevenson, Miss H. C., 11 Fosse Way, Ealing, London, W. 13. (1947.)
Tipler, Mrs M. K., B.Sc., Imperial Chemical Industries Ltd., Hawthorndale Laboratories, Jealott's Hill Research Station, Bracknell, Berks. (1945.)
Tomlinson, J., B.Sc., Botanical Department, The University, Edgbaston, Birmingham 15. (1946.)
Vachell, Miss E., F.L.S., Fairfield, Llandaff, Cardiff. (1945.)
Whitehouse, H. L. K., The Botany School, Cambridge. (1946.)
Wilman, Miss A. M., B.Sc., Royal Holloway College, Englefield Green, Surrey. (1947.)
Winfield, Miss J., B.Sc., Botany Department, Imperial College of Science and Technology, South Kensington, London, S.W. 7. (1946.)

Members are requested to notify the Treasurer (W. Buddin) and the Secretary (C. J. Hickman) of any change of address

RULES

Society's Name and Object

1. The Society shall be called 'The British Mycological Society', and its object shall be the study of Mycology in all its branches.

Members of Society

2. The Society shall consist of Honorary Members, Members (including Foundation Members*), and Associates; the number of Honorary Members shall be limited to 20 at any one time but the numbers of Members and Associates shall be unlimited.

Honorary Members

3. Honorary Members shall be persons of pre-eminence in Mycology, or who have rendered special service to the Society.

Officers

4. The Officers of the Society shall consist of a President, two or more Vice-Presidents, Treasurer, Secretaries and Editor or Editors. They shall be elected annually, at the Annual General Meeting of the Society.

Government of Society

5. The government of the Society shall be vested in a Council, which shall consist of the President and other Officers, together with nine other Members who shall be elected annually at the Annual General Meeting, three of whom shall be elected to replace three retiring members, who shall not be eligible for immediate re-election. The Members to retire shall be those who have been longest in office, or, where there is equality, those determined by ballot. Ex-Presidents who have served their year of office prior to 31 December 1936 are *ex-officio* members of the Council. From 1937 the retiring President shall become a Vice-President for a period of two years.

Period of Office

6. The Officers and Council shall hold office from the 1st of January following their election.

Plant Pathology Committee

7. The special interests of Plant Pathology shall be delegated to an executive committee, to be called the Plant Pathology Committee of the British Mycological Society. The Committee shall consist of the President and Secretaries *ex-officio* and twelve other Members of the Society. The latter shall be elected annually at the Annual General Meeting, and three shall retire each year and shall not be eligible for immediate re-election.

* Foundation Members are those Members or Societies who joined the Society previous to the limit of 100 Members having been attained. This was reached 22 October 1903.

The Members to retire shall be those who have been longest in office, or, where there is equality, those determined by ballot.

The Officers shall consist of a Chairman and a Secretary, to be elected by the Committee each year.

At least two meetings shall be held every year, six members to form a quorum.

The Committee shall have power to appoint for any special purpose a sub-committee consisting either wholly or partly of members of the Committee.

Election of Members

8. Honorary Members shall be elected only at a meeting of the Society by a majority of the Members then present.

Every candidate for election as a Member shall be proposed by two Members, who shall sign a certificate (see Appendix) of recommendation, one at least of the proposers so certifying from personal knowledge. Each candidate shall sign an undertaking to abide by the Rules if elected (see Appendix). They shall be elected by a majority of the Members present at any meeting of the Society or by the President and Officers of the Society.

Subscription

9. All Members shall pay an annual subscription of one pound, Foundation Members paying five shillings, due on the 1st of January in each year. Honorary Members shall be exempt from any such payment.

A Member wishing to retire from the Society shall give notice to the Secretary or Treasurer in writing before the 1st of December of the previous year.

Associates

10. All Associates shall be proposed by two Members. They shall be elected by a majority of the Members present at a meeting of the Society and shall pay an annual subscription of five shillings. They shall be eligible to attend the Society's meetings and forays but not to vote at any meeting or to receive the Transactions.

Meetings

11. The Society shall hold one or more meetings annually, at a place and time determined by the Members at the preceding Annual General Meeting, or by the Council.

Accounts

12. At the Annual General Meeting in each year the Treasurer shall present duly audited accounts.

Alteration of Rules

13. The Rules shall not be altered except by a two-thirds majority of the Members present at an Annual General Meeting. A printed copy shall be sent to every Member on election, and, in the event of alterations, to all Members.

APPENDIX

Form of proposal for Membership or Associateship of the British Mycological Society

.....

of .....

.....

being desirous of becoming a of the British Mycological Society, we, the undersigned Members of the Society, certify that we consider h to be a desirable of the Society, and beg to recommend h for election.

Dated this day of 19

.....(From personal knowledge.)

.....

Certificate to be signed by the Candidate

I hereby certify that I desire to become a of the British Mycological Society and that I will abide by the Rules if elected.

.....

EDITORIAL NOTE

The Editors have found it necessary, in the preparation of the Jubilee Volume, to depart at times from the customs ordinarily followed in producing the *Transactions*. The usual customs will be observed in subsequent volumes.

INDEX

New names are indicated by an asterisk.

Absidia van Tiegh., 130
  cylindrospora Hagem, 107, 109–12
  glauca Hagem, 107, 109–12
  orchidis (Vuill.) Hagem, 109, 110
Acremoniella atra Sacc., 111
Acrostalagmus albus Preuss, 109
Acrothecium Corda, 107
Actinomyces antibioticus (Waksman & Woodruff) Bergey *et al.*, 60
  bovis Harz, 44, 48
  griseus Krainsky, 60
  lavendulae Waksm. & Curt., 60
actinomycosis, 44, 48
Agaricus L. ex Fr., 153
Aleurodiscus Rabenh., 155
Alternaria Nees ex Wallr., 72
  radicina Meier, Drechsl. & Eddy, 72
Amanita (Pers. ex Fr.) S. F. Gray, 93–5, 175, 176
  muscaria (Linn.) Fr., 94, 95
  porphyria (Alb. & Schw.) Fr., 95
Anthostomella pullulans (de Bary) Bennett, 142
Apium graveolens (celery), 67
Arcangeliella Cav., 161, 164
Armillaria (Fr.) Quél., 9, 175
  mellea (Vahl ex Fr.) Quél., 32, 103
Ascochyta Lib., 68, 69
  boltshauseri Sacc., 69
  phaseolorum Sacc., 69
  pinodella L. K. Jones, 69
  pisi Lib., 68, 69
Ascoidea rubescens Brefeld, 121, 123
aspergillosis, 47
Aspergillus Mich. ex Fr., 47, 118
  clavatus Desm., 60
  flavus Link, 60
  flavus-oryzae (group), 59, 60
  fumigatus Fres., 47, 48, 55, 60
  giganteus Wehmer, 60
  glaucus Link ex Fr., 55
  niger van Tiegh., 29, 47, 50, 53, 54, 118, 123, 124
  parasiticus Speare, 60
  terreus Thom, 60, 130
  ustus (Bainier) Thom & Church, 60
Asterodon Pat., 157
Asterostroma Massee, 157
athlete's foot, 40
Avena (oat), 75, 80

Battarraea Pers., 166
  guicciardiniana Cesati, 166
Beta (beet), 68, 71, 72
Betula (birch), 92, 94
bios, 118
Birkinshaw, J. H., 'Some aspects of fungal metabolism: with particular reference to the production of antibiotics', 20, 50
Blastomyces Cost. & Roll., 47
  dermatitidis Gilchrist & Stokes, 46
blastomycosis, 43, 44, 46, 47
Bolbitius Fr., 166
Boletinus Kalchbr., 93
Boletopsis Fayod., 158
Boletus Dill. ex Fr., 93, 94, 123, 168, 173–5
  elegans (Schum.) Fr., 94
  granulatus (L.) Fr., 93, 95, 122
  luteus (L.) Fr., 93
  pinicola (Vitt.) Rea, 4
  sulphureus Fr., 4, 184
  variegatus (Swartz) Fr., 93, 95
  xerocomi, 173
Botryotinia Whetzel, 87, 88
Botrytis Pers. ex Fr., 74, 85–8, 90, 103
  antherarum trifolii Schlecht, 86
  anthophila Bondarzew, 84, 86–9
  cinerea Pers. ex Fr., 77, 87–9, 106, 111
    f. lini van Beyma, 72
  trifolii van Beyma, 86
Brachycladium Corda, 113
  penicillatum Corda, 117
  ramosum Bainier, 113, 114, 117
  ramosum (Cooke) A. L. Smith, 113
Brassica, 72
Brett, M. A., 'The problem of *Cladosporium herbarum*', 21, 141
British Mycological Society, by J. Ramsbottom, 1
Byssocorticium Bondarzew & Singer, 158

Cadophora americana Nannf., 47
Calodon (Quél.) Karst., 174
Campanella P. Henn., 158
Candida albicans (Robin) Berkh., 48
Cantharellus Adans. ex Fr., 93, 155, 176
Carleton Rea, by J. Ramsbottom, 180
Cenococcum graniforme (Sow.) Ferdinand. & Winge, 94
Cephalosporium Corda, 103
  acremonium Corda, 109
  asperum Marchal, 107
Chesters, C. G. C., 'A contribution to the study of fungi in the soil', 20, 100
chromoblastomycosis, 46, 48
Circinella van Tiegh. & Le Monnier, 103, 106
Citromyces Wehmer, 29, 50
Cladosporium Link ex Fr., 141–4, 146–9
  herbarum Link ex Fr., 21, 109–12, 141–4, 147, 148, 150
Clathrogaster Petri, 164
  vulvarius Petri, 163
Clavaria Vaill. ex Fr., 21, 153, 154, 158
  cinerea (Bull.) Fr., 154
  dendroidea Jungh., 121
  luteoalba Rea, 184
Claviceps (ergot), 67
Clavulina Schroet., 155
Clitocybe (Fr.) Quél., 94
  candida Bres., 59
  maxima (Fr.) Quél., 59

Clitopilus (Fr.) Quél., 93
prunulus (Scop.) Fr., 95
Clivia, 176
coccidioidal granuloma, 41
Coccidioides Stiles, 43, 46, 48, 49
immitis Stiles, 45, 48
coccidioidomycosis, 41–4, 49
Colletotrichum Corda, 69, 74
lindemuthianum (Sacc. & Magn.) Bri. & Cav., 67–9
linicola Pethybr. & Laff., 72, 77, 81
Collybia (Fr.) Quél., 94, 169, 171
platyphylla (Pers.) Fr., 4
Coniophora cerebella Pers., 53
laeticolor (Karst.) Karst., 155
Coniophorella Karst., 155
Conocybe Fayod, 166
Coprinus (Pers. ex Fr.) S. F. Gray, 166
Coriolus Quél., 158
abietinus (Dicks.) Quél., 175
versicolor (L. ex Fr.) Quél., 175
Corticium Pers. ex Fr., 155, 156, 157
archeri Berk., 155
chrysocreas B. & C., 155
coronilla v. Hoehn. & Litsch., 153
croceum (Kunze) Bres., 93
polygonium (Pers.) Fr., 155
sulphureum Fr., 93
sulphureum Pers. non Fr., 155
tulasnelloideum v. Hoehn. & Litsch., 155
Cortinarius (Pers. ex Fr.) S. F. Gray, 93, 94, 181
caerulescens Fr., 181
Council, Members of, 1896–1946, 14
Craterellus Pers., 155
Cryptococcus Kuetzing, 43
Cunninghamella Matr., 103
elegans Lendner, 112
Curvularia Boedijn, 113, 117
inaequalis (Shear) Boedijn, 117
ramosa (Bainier) Bodeijn, 113, 114, 117
Cyphella Fr., 158, 174
Cyttarophyllum Heim, 166
besseyi (Peck) Heim, 167

Daedalea Pers. ex Fr., 157, 158
biennis (Bull.) Quél., 157
confragosa (Bolt.) Fr., 158
quercina Fr., 157
Daucus (carrot), 72
Dematium Pers. ex Fr., 141, 142
pullulans de Bary, 141–3, 150
Dendryphion, 113
ramosum (Bainier) Sacc. & Trav., 113, 114
Dendryphium ramosum Cooke, 113
dermatophytes, 45
dermatophytosis, 43–5
Dermocybe sanguinea (Wulf. ex Fr.) Ricken, 55
desert rheumatism, 41
Dictyoploca Mont., 169
guadalupensis Heim, 172
heterophylla Heim, 169, 170, 172
myrmecophila Heim, 169, 170, 172
Dodgea Malençon, 168
occidentalis Malençon, 167
Doyer, L. C., 'Several seed-borne diseases and methods for identifying them in seed testing', 20, 67

Editorial note, 202
Editors, 1896–1946, 14
Effect of certain growth substances on mycelial growth and fruiting of *Melanospora destruens* Shear, by L. E. Hawker, 21, 135
Elasmomyces Cav., 161, 163, 164, 174
krjukowensis Buch., 163
mattirolianus Cav., 163, 167
russuloides Setchell, 164
Emmons, C. W., 'Medical mycology', 20, 40
Entoloma (Fr.) Quél., 93
griseocyaneum Fr., var. roseum Maire, 3
Epidermophyton Sabour., 45
expansin, 59

Fagus (beech), 92
Favolaschia Pat., 158, 171
calocera Heim, 171
favus, 40, 45
film, Predacious fungi, 20
Fistulina Bull. ex Fr., 158
Fomes (Fr.) Kickx, 157, 159
annosus Fr., 123
fomentarius (L.) Fr., 157
laevigatus Corner, 159
laricis (Jacq.) Murr., 53
Forays, lists of, 15
Fries, N., 'The nutrition of fungi from the aspect of growth factor requirements', 21, 118
Fungal metabolism, with particular reference to the production of antibiotics, by J. H. Birkinshaw, 50
Fungi in the soil, a contribution to the study of, by C. G. C. Chesters, 20, 100
Fusarium Link ex Fr., 52, 67, 70, 71, 74, 103, 104, 107, 111, 112
avenaceum (Fr.) Sacc., 70, 122
culmorum (W. G. Sm.) Sacc., 70
herbarum (Corda) Fr., 70
Fusarium javanicum Koord., 60
lini Bolley, 77

Galera (Fr.) Quél., 166
Ganoderma Karst., 159
applanatum (Pers. ex Wallr.) Pat., 153, 159
lucidum (Leyss. ex Fr.) Karst., 159
Gastrodia, 9
General Secretaries, 1896–1945, 14
Gibberella Sacc., 71
zeae (Schw.) Petch, 71
Gilchrist's disease, 44, 46
Gladiolus, 176
Gliocladium fimbriatum Gilman & Abbott, 60
Gloeocystidium v. Hoehn., 155
Gomphidius Fr., 93
Grandinia Fr., 155, 156
Gymnomyces Massee & Rodway, 164
Gyrodon Opat., 174
Gyrophragmium Mont., 166, 174
delilei Mont., 167

Haliotis, 147
Haplosporangium parvum, 48
Hawker, L. E., 'The effect of certain growth substances on mycelial growth and fruiting of *Melanospora destruens* Shear', 21, 135
Hebeloma (Fr.) Quél., 93
Heim, R., 'Phylogeny and natural classification of macro-fungi', 21, 161
Helminthosporium Link ex Fr., 71, 74–6, 80, 114
avenae (B. & Br.) Eidam, 75, 76, 81, 82
*biforme Mason & Hughes, n.sp., 107, 109, 112, 114
cynodontis Marignoni, 55
gramineum Rabenh., 55, 71
sativum Pamm. King & Bakke, 71
teres Sacc., 71
Hemileia vastatrix B. & Br., 29
Hemimycena Singer, 169
Hexagona Fr., 158
dybowskii Pat., 156
hirta (Pal.) Fr., 156
hystrix (Cooke) Har. & Pat., 156
Hirschioporus Donk, 158
Histoplasma Darling, 43
capsulatum Darling, 46
histoplasmosis, 42, 46
Honorary members, 1896–1946, 15
Hordeum (barley), 69, 71
Hormodendron Bon., 141–50
cladosporioides Sacc., 141, 143
Hormodendrum pedrosoi Brumpt, 47
Hyalopus Corda, 103
Hydnangium Wallr., 164
Hydnochaete Bres., 157
Hydnocystis Tul., 168
Hydnum L. ex Fr., 93, 158
farinaceum Pers., 155
imbricatum Fr., 5
Hygrophorus Fr., 93, 162
citrinus Rea, 3
reai Maire, 3, 184
Hymenochaete Lév., 157
Hypochnus Fr. em. Karst., 155
fumosus Fr., 32, 155
Hypomyces chrysospermus (Bull.) Tul., 168

Inocybe (Fr.) Quél., 93
Irpex Fr., 158
fuscoviolaceus Fr., 175
Ixechinus Heim, 174

Jubilee Meeting, London, 20–25 October 1946, 17

kala azar, 46
Kneiffia chromoplumbea B. & Br., 155
wrightii B. & C., 155

Lachnea (Fr.) Gill., 168
Lactariopsis P. Henn., 163, 164
pandani Heim, 163
Lactarius Pers. ex S. F. Gray, 93, 94, 161–4
adhaerens Heim, 163, 164
deliciosus (L.) Fr., 94, 95, 98
gymnocarpus Heim, 162
melanogalus Heim, 162
rubroviolaceus, 162
theiogalus Fr., 2
Lactuca (lettuce), 85
Larix (larch), 94
Laschea clypeata Pat., 169
Leishmania, 46
Lentinus Fr., 174
Lenzites Fr., 157, 158
tricolor (Bull.) Fr., 158, 174
Lepiota (Pers. ex Fr.) S. F. Gray, 35, 175
Leptonia reaae Maire, 3
Leptosphaeria tritici (Garovaglio) Pass., 142
Leptotus Karst., 158
Linum (flax), 72, 75, 77, 80, 81
List of Members, 186
Lolium (ryegrass), 75, 78, 80, 81, 88
Lophodermium pinastri (Schred.) Chev., 121
Lycoperdon perlatum Pers., 21

Maccagnia Mattir., 164
Macowanites Kalchbr., 164
agaricinum Kalchbr., 167
Macrosporium Fr., 70
commune Rabenh., 70
Marasmius Fr., 94, 123, 171
perforans Fr., 122
Martellia Mattir., 161, 164
Medicago (alfalfa), 85, 86
Medical mycology, by C. W. Emmons, 20, 40
Melanospora Corda, 135, 136
destruens Shear, 21, 121, 135–9
Melilotus (sweet clover), 86
Melin, E., 'Recent advances in the study of tree mycorrhiza', 20, 92
Members, list of, 186
Merisma Pers. ex S. F. Gray, 158
Metarrhizium glutinosum Pope, 60
Microsporum Gruby, 45
Monilia Pers. ex Fr., 78
fructigena Pers., 135
moniliasis, 43
Montagnites Fr., 166
Moore, W. C., 'Mycology and plant pathology', 64
Mortierella Coemans, 103, 105, 106
baineri Cost., 107, 109–11
candelabrum van Tiegh. & Le Monnier, 111
canina Dauph., 107
humilis Linneman, 107, 109–12
pilulifera van Tiegh., 112
polycephala Coemans, 111
pusilla Oudemans, 112
renispora Dixon-Stewart, 105, 106
simplex van Tiegh. & Le Monnier, 107, 109–11
Mucor Mich. ex Fr., 103–6
hiemalis Wehmer, 105–7, 109–12, 136
ramannianus Moeller, 107, 109–12, 124
silvaticus Hagem, 111
Muskett, A. E., 'Technique for the examination of seeds for the presence of seed-borne fungi', 20, 74
Mycena (Fr.) S. F. Gray, 94, 153, 169, 171, 175, 176

Mycena (*continued*)
decaryi Heim, 172
longicystis Heim, 169, 172
pseudopura Cooke, 169, 171
Mycenoporella van Overeem, 169
clypeata Pat., 170, 172, 173
lutea van Overeem, 172
Mycobacterium tuberculosis (Schroet.) Lehm. & Neum., 58
Mycology and plant pathology, by W. C. Moore, 64
Mycomedusa Heim, 171
guineensis Heim, 171, 173
Mycosphaerella pinodes (Berk. & Blox.) Vesterg., 68, 69
tulasnei Jancz., 142

Nectria cinnabarina (Tode ex Fr.) Fr., 21
Nematospora gossypii Ashby & Nowell, 120, 121, 137, 138
Nephromopsis endocrocea, 55
Neurospora Shear & Dodge, 62, 126, 128–32
crassa Shear & Dodge, 129
sitophila Shear & Dodge, 78
Noble, M., 'Seed-borne diseases of clover', 20, 84
Nocardia asteroides (Eppinger) Blanchard, 48
Nutrition of fungi from the aspect of growth factor requirements, by N. Fries, 21, 118

Odontia Pers. ex S. F. Gray, 155
conspersa Bres., 155
hydnoides v. Hoehn. & Litsch., 155
Omphalia (Fr.) S. F. Gray, 171
alleni Maire, 3
Oospora colorans, 55
Ophiobolus Riess, 89
Ophiostoma Syd., 120, 122, 123, 130–2
catonianum Goidanich, 121
multiannulatum (Hedgcock & Davidson) Hendrickx, 130
ulmi (Buism.) Nannf., 122, 123

Paecilomyces elegans (Corda) ? Thom, 109, 111, 112
paracoccidioidal granuloma, 44
Parmelia caperata Ach., 53
Past-Presidents and other Officers, Members of Council, and Honorary Members, together with list of the Autumn and Spring Forays, 1896–1946, 13
Paxillus Fr., 93, 168, 171, 173
involutus (Batsch) Fr., 168
panuoides Fr., 173
penicillin, 56–8, 61, 62
Penicillium Link ex Fr., 29, 50, 78, 103 105, 107, 109, 110, 112, 118, 135
aurantio-virens Biourge, 60
brevicompactum Dierckx, 60
charlesii G. Smith, 54
chrysogenum Thom, 60, 62, 130
citreo-roseum Dierckx, 55
citrinum Thom, 60
cladosporioides Fres., 141
cyclopium Westling, 54, 55, 60
expansum (Link) Thom, 59, 60
gladioli Machacek, 60
glaucum Link, 50
luteum Zukal, 111
minioluteum Dierckx, 53
nigricans Bainier, 111, 112
Penicillium notatum Westling, 56, 57, 60
patulum Bainier, 60
phoeniceum van Beyma, 55
puberulum Bainier, 60
resticulosum Birkinshaw, Raistrick & G. Smith, 60
spiculisporum Lehman, 53
spinulosum Thom, 55, 60
terrestre Jensen, 54
Peniophora Cooke, 155
byssoidea (Pers.) von Hoehn. & Litsch., 155
dryina (B. & C.) Rogers & Jackson, 155
hydnoides Cooke & Massee, 155
Pestalozzia hartigii Tubeuf, 136
Phaeomycena Heim, 169
aureophylla Heim, 170, 172
Phallus imperialis Schulz., 184
Phaseolus vulgaris (bean), 67–70
Phialea (Fr.) Gill., 74
temulenta Prill. & Delacr., 78, 79, 81, 82, 88
Phialophora Thaxt. in Medlar, 47, 48
verrucosa Medlar, 47
Phlebia vaga Fr., 155
Phlebomycena Heim, 169
medecassensis Heim, 169, 170, 172
Phlebopus (Heim) Singer, 174
Phoma Desm., 72, 74, 77, 81, 103, 112
betae (Oudem.) Frank, 68, 71
lingam (Fr.) Desm., 72
Phycomyces Kunze ex Fr., 119, 120, 121, 128
blakesleeanus Burgeff, 121
Phylloporus Quél., 171, 173
Phylogeny and natural classification of macrofungi, by R. Heim, 21, 161
Phytophthora de Bary, 124
Picea (spruce), 92–4, 96
Pinus (pine), 32, 92–4, 96, 97
strobus, 98
sylvestris, 94
Pisum (pea), 68
Pityrosporum ovale Castellani & Chalmers, 119, 120
Pleospora herbarum (Fr.) Rabenh., 70
Podaxon Fr., 166
Polyporus Mich. ex Fr., 157, 174, 175
adustus (Willd.) Fr., 137
berkeleyi Fr., 158
cristatus (Pers.) Fr., 158
giganteus (Pers.) Fr., 158
leucomelas (Pers.) Fr., 158
ovinus (Schaeff.) Murrill, 158
scobinaceus (Cumino) Bres., 174
Polyspora lini Pethybr. & Laff., 72, 77, 81
Polystictus Fr., 157, 158
abietinus (Dicks. ex Fr.) Fr., 158
floccosus (Jungh.) Fr., 156
xanthopus Fr., 159
Populus (aspen), 94
Poria Pers. ex S. F. Gray, 156
onusta (Karst.) Bres., 156

Porolaschia Pat., 171
Poromycena van Overeem, 169
brunnea van Overeem, 172
decipiens van Overeem, 169, 170, 172
manipularis (Berk.) van Overeem, 169, 170, 172
Porothelium Fr., 158
predacious fungi, film of, 20
President, C. G. C. Chesters, 19
Presidential address, by J. Ramsbottom, 22
Presidents, list of, 1896–1946, 13
Problem of *Cladosporium herbarum*, by M. A. Brett, 21, 141
Protoglossum Massee, 164
luteum Massee, 167
Psalliota (Fr.) Quél., 153, 154, 176
arvensis (Schaeff.) Fr., 153, 154
augusta Fr., 153, 154
campestris (L.) Fr., 153
haemorrhoidaria Kalchbr., 153
silvicola (Vitt.) Fr., 153 154
villatica Brondeau, 154
xanthoderma Genev., 153, 154
Pseudotrametes Bondarzew & Singer, 159
gibbosa (Pers.) Bondarzew & Singer, 159
Pullularia pullulans (de Bary) Berkhout, 77, 111, 112, 142, 150
Pyronema confluens (Pers.) Tul., 106, 119, 120
Pythium Pringsh., 59, 103–5
artotrogus (Mont.) de Bary, 103
debaryanum Hesse, 59
intermedium de Bary, 103, 106
mamillatum Meurs, 103
ultimum Trow, 103, 105, 106

Queletia Fr., 166
Quercus (oak), 92

Ramsbottom, J., 'Carleton Rea', 180
'Closing remarks', 179
'Presidential Address', 22
'The British Mycological Society', 1
Rhizoctonia DC. ex Fr., 103
solani Kuehn, 103, 104, 106, 112
Rhizopogon Fr. & Nordh., 94, 168
Rhizopus Ehrenb. ex Corda, 103
Rhodophyllus Quél., 168
Rhodotorula aurantiaca (Saito) Lodder, 119, 120
Richoniella Cost. & Dufour, 168
leptoniaespora (Richon) Cost. & Dufour, 168
ringworm, 40, 43, 45
Rules, 199
Russula Pers. ex S. F. Gray, 93, 94, 181, 161–4
adusta (Pers.) Fr., 162
annulata Heim, 163
fragilissima Heim, 162
luteostacta Rea, 181
nigricans (Bull.) Fr., 162
radicans Heim, 163, 167

Saccharomyces Meyen, 52, 122
Schizophyllum Fr., 174
Scleroderma Pers., 94
Sclerotinia Fuckel, 67, 84, 85, 87, 88, 89
bifrons Seaver, 88
minor Jagger, 84, 85, 86, 90
sativa Drayton & Groves, 84–6
*spermophila Noble, n.sp., 84–90
trifoliorum Erikss., 84–6, 89, 90, 135
Sclerotium roseum Moug. ex Fr., 4
Sebacina Tul., 32
Secale (rye), 71
Secotium G. Kunze, 166
malinvernianum Cesati, 167
Secretaries, 1896–1946, 14
Seed-borne diseases of clover, by M. Noble, 20, 84
Seed-borne fungus diseases and methods for identifying them in seed testing, by L. C. Doyer, 20, 67
Septoria Sacc., 74
apii Chester, 67
Sistotrema Pers. ex Fr., 156, 158
confluens Pers., 156
Solenia Pers. ex Fr., 174
Sordaria Ces. & de Not., 103
fimicola (Rob.) Ces. & de Not., 112
Sparassis crispa (Wulf.) Fr., 33
Sphaeropsis malorum Peck, 135
sporotrichosis, 44, 45, 48
Sporotrichum Link ex Fr., 43, 45, 111
schenkii Hekt. & Perk., 44, 45
Staphylococcus aureus Rosenbach, 61
Stemphylium Wallr., 72
radicinum (Meier, Drechsl. & Eddy) Neergaard, 72
Stephensia Tul., 168
Stereum Pers. ex S. F. Gray, 157
pini Fr., 155
rufum Fr., 155
Streptomyces griseus (Krainsky) Waksman & Schatz, 58
lavendulae (Waksman & Curtis) Bergey *et al.*, 58
streptomycin, 58, 61
Strobilomyces Berk., 174
cinnabarinus Heim, 167

Taxonomic problems in Hymenomycetes, by E. M. Wakefield, 21, 152
Technique for the examination of seeds for the presence of seed-borne fungi, by A. E. Muskett, 20, 74
Termitomyces Heim, 175
Thelephora Ehrh. ex Fr., 155, 158
thrush (disease), 48
Tilia (lime), 94
Tilletia caries (DC.) Tul., 67, 119, 120
Tomentella Pat., 155, 158
Torula Pers. ex Fr., 47
Trametes Fr., 158, 159
cristata Cooke, 156
hydnoides Fr., 156
rubescens (Alb. & Schw.) Fr., 158, 175
suaveolens (L.) Fr., 159
Treasurers, 1896–1946, 14
Trechyspora Karst., 156, 158
Tree mycorrhiza, recent advances in the study of, by E. Melin, 20, 92

Tremellodon gelatinosum (Scop.) Fr., 35
Trichoderma Pers. ex Fr., 105
  viride Pers. ex Fr., 60, 105, 106, 107, 109–12
Tricholoma (Fr.) Quél., 93, 94, 176
  brevipes (Bull.) Fr., 94
  fumosum (Pers. ex Fr.) Ricken, 94
  nudum (Bull.) Fr., 94
  personatum Fr., 94
Trichophyton Malmsten, 45
  discoides Sabouraud, 120, 121
  mentagrophytes (Robin) Blanchard, 48
  rubrum Castellani, 44
Trifolium pratense (red clover), 84–6, 89
  repens (white clover), 84, 87–90
Triticum (wheat), 67, 69, 71
Truncocolumella Zeller, 168
Tuber Mich. ex Fr., 168
Tulostoma Pers., 166
Typhula (Pers.) Fr., 89
Ulex minor, 144, 149, 150
Urtica dioica, 114
Ustilago nuda (Jens.) Rostr., 69
  tritici (Pers.) Rostr., 69
  violacea (Pers.) Wint., 122

vaginitis, 48
valley fever, 41
Venolactarius adhaerens Heim, 162
Vice-Presidents, 1896–1946, 13
Volvaria (Fr.) Quél., 175

Wakefield, E. M., 'Taxonomic problems i
  Hymenomycetes', 21, 152

Xanthoria parietina Th. Fr., 55
Xerocomus Quél., 173, 175

Zygorhynchus Vuill., 103
  moelleri Vuill., 107, 109–12

THE BRITISH MYCOLOGICAL SOCIETY'S TRANSACTIONS

VOLUME

I. Pt. I (out of print); II, 1897–98 (5/6); III, 1898–99 (5/6); IV (out of print); V, 1900–01 (out of print).

II. Pt. I, 1902 (11/6); II, 1903 (11/6); III, 1904 (15/-); IV, 1905 (15/-); V, 1906 (15/-).

III. Pt. I, 1907 (15/-); II, 1908 (16/-); III, 1909 (16/-); IV, 1910 (16/-); V, 1911 (16/-).

IV. Pt. I, 1912 (16/-); II (out of print).

V. Out of print.

VI. Pts. I & II (out of print); III, 1919 (8/-); IV, 1919 (10/6).

VII. Pts. I & II*, 1920 (12/6); III, 1920 (12/6); IV, 1921 (12/6).

VIII. Pts. I & II*, 1922 (15/-); III, 1923 (7/6); IV, 1923 (7/6).

The University Library,
ALLAHABAD.
T.V.
Accession No. 114210 Botany.
Call No. 5805—13
30
(Form No. 28 L 20,000—'49.)

Double numbers are indicated by an asterisk.

The volumes and parts which are out of print are indicated in their proper places above. Owing to the scarcity of some of the other parts, those parts cannot be sold except in complete volumes. Long runs, including volumes or parts out of print, are sometimes available.

From Volume VIII inclusive, the prices of all back numbers have been increased by 33⅓%. The prices listed above will therefore be increased forthwith.

Enquiries and orders to be addressed to

CAMBRIDGE UNIVERSITY PRESS
Bentley House, 200 Euston Road, London, N.W. 1

CONTENTS

	PAGE
The British Mycological Society. By J. Ramsbottom	1
Past-Presidents and other officers, Members of the Council, and Honorary Members, together with lists of the Autumn and Spring Forays 1896–1946	13
The Jubilee Meeting, London, 20–25 October 1946	17
Presidential Address. By J. Ramsbottom	22
Medical mycology. By C. W. Emmons	40
Some aspects of fungal metabolism; with particular reference to the production of antibiotics. By J. H. Birkinshaw (with 7 Text-figures)	50
Mycology and plant pathology. By W. C. Moore	64
Several seed-borne fungus diseases and methods for identifying them in seed testing. By Lucie C. Doyer	67
Technique for the examination of seeds for the presence of seed-borne fungi. By A. E. Muskett (with Plates I—III)	74
Seed-borne diseases of clover. By Mary Noble (with Plates IV and V)	84
Recent advances in the study of tree mycorrhiza. By Elias Melin	92
A contribution to the study of fungi in the soil. By C. G. C. Chesters (with 5 Text-figures)	100
The nutrition of fungi from the aspect of growth factor requirements. By Nils Fries (with 5 Text-figures)	118
The effect of certain growth substances on mycelial growth and fruiting of *Melanospora destruens* Shear. By Lilian E. Hawker (with Plates VI and VII)	135
The problem of *Cladosporium herbarum*. By Margaret A. Brett (with Plate VIII and 2 Text-figures)	141
Taxonomic problems in Hymenomycetes. By Elsie M. Wakefield	152
Phylogeny and natural classification of macro-fungi. By Roger Heim (with 19 Text-figures)	161
Closing remarks. By The President	179
Carleton Rea. By J. Ramsbottom	180
List of Members	186
Rules	199
Editorial Note	202
Index	203

LIST OF COMMON
BRITISH PLANT DISEASES
Compiled by the
Plant Pathology Committee of the British Mycological Society
Third Edition. Revised and Enlarged. Reprinted 1945
5*s. net. Postage* 3*d.*

COOKE'S
ILLUSTRATIONS OF BRITISH FUNGI
Annotations by L. QUÉLET, R. MAIRE and C. REA
Preface by A. A. PEARSON
Reprinted from the Transactions of the British Mycological Society
Vol. XX. Part 1. August 1935
2*s.* 6*d. net. Postage* 3*d.*

BRITISH HYPOCREALES, by T. PETCH
Reprinted from the Transactions of the British Mycological Society
Vol. XXI. June 1938.
2*s. net. Postage* 2*d.*

CAMBRIDGE UNIVERSITY PRESS

Printed in Great Britain at the University Press, Cambridge (Brooke Crutchley, University Printer)